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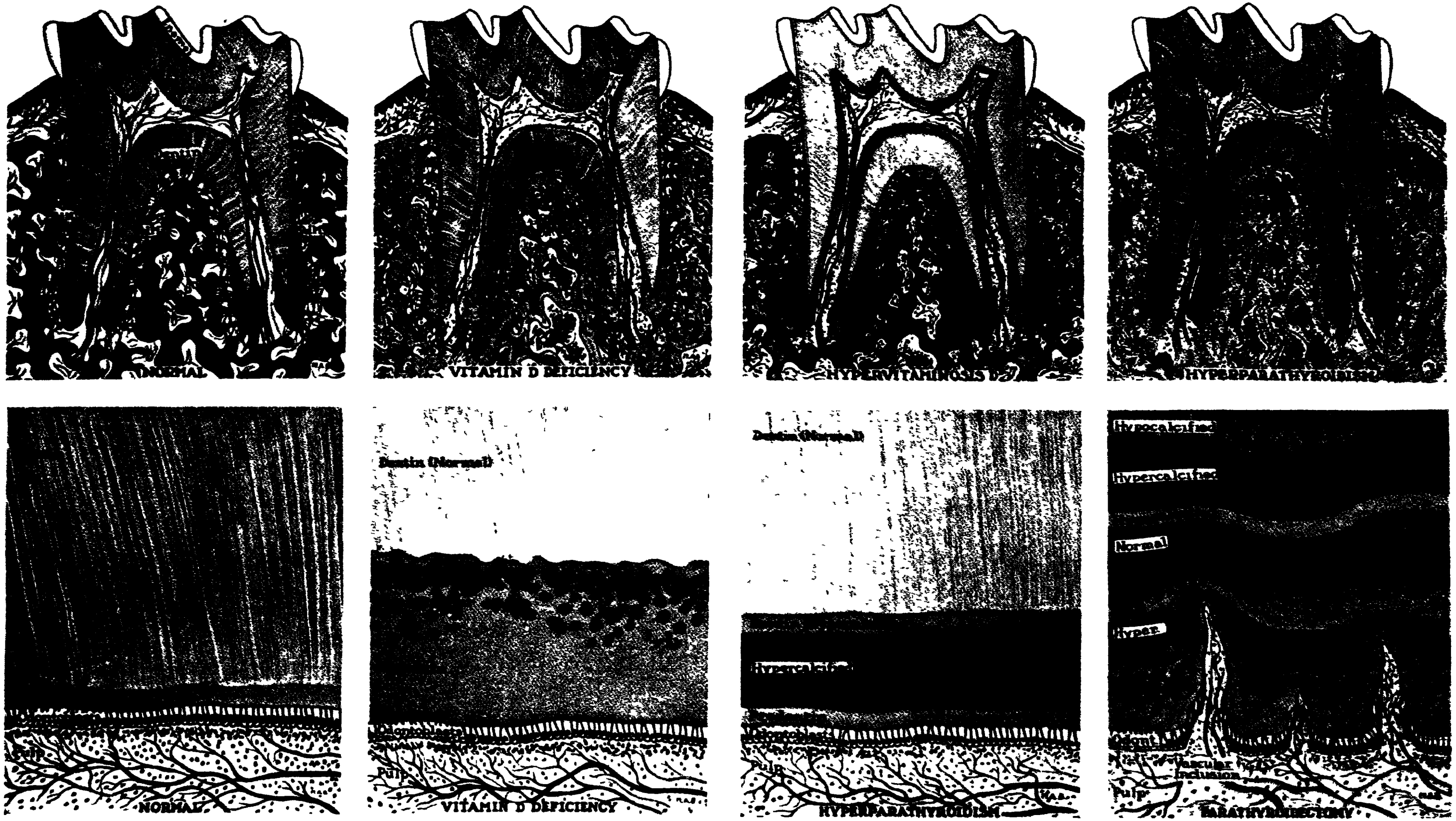

Plate 1. Experimental Changes in the Teeth of the Rat as seen in Decalcified Sections Stained with Hematoxylin and Eosin.

Plate 1. Experimental Changes in the Teeth of the Rat. Top Row, Molar; Bottom Row, Dentin of Incisor.

TOP ROW

Normal. The characteristic experimental changes in the periodontal tissues of the rat molar.

Vitamin-D Deficiency. The bone and cementum are *undercalcified* and hyperplastic, with resulting ankylosis.

Hypervitaminosis D. The bone and cementum are *overcalcified* and hyperplastic, with resulting ankylosis.

Hyperparathyroidism. The alveolar bone (red) shows marked resorption and osteoclasis with replacement by fibrous tissue. Note the absence of resorption of dentin.

BOTTOM ROW

Normal. The characteristic experimental changes in the growing dentin of the rat incisor.

Vitamin-D Deficiency. The dentin is undercalcified. Note the wide predentin and the markedly interglobular dentin.

Hypervitaminosis D (or *Hyperparathyroidism*). Note the primary *hypo*calcified and secondary *hyper*calcified zone in the dentin.

Parathyroidectomy. The dentin is poorly calcified. Note the alternate stratification of over- and undercalcified layers and the vascular inclusions in the dentin.

See text of Chapter 6 for further descriptive details.

THE RAT
IN
LABORATORY INVESTIGATION

By a Staff of Twenty-Nine Contributors

EDITED BY

EDMOND J. FARRIS, PH.D.
Associate Member in Anatomy, Executive Director
The Wistar Institute of Anatomy and Biology

AND

JOHN Q. GRIFFITH, JR., M.D.
John Q. Griffith, Jr., Research Foundation, Philadelphia
Formerly Associate in Medicine, University of Pennsylvania

179 ILLUSTRATIONS

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THE AUTHORS

WILLIAM H. F. ADDISON, B.A., M.D.
Professor Emeritus of Histology and Embryology,
School of Medicine, University of Pennsylvania

C. JELLEFF CARR, PH.D.
Associate Professor of Pharmacology, School of Medicine,
University of Maryland

ADOLPH J. CRESKOFF, A.B., M.D.
Associate in Medicine, School of Medicine,
University of Pennsylvania

EDMOND J. FARRIS, PH.D.
Associate Member in Anatomy, Executive Director,
The Wistar Institute of Anatomy and Biology

THOMAS FITZ-HUGH, JR., A.M., M.D.
Associate Professor of Clinical Medicine,
School of Medicine, University of Pennsylvania

WILFRED E. FRY, M.D.
Professor of Ophthalmology, Graduate School of Medicine,
Assistant Professor of Medicine, School of Medicine,
University of Pennsylvania
Surgeon, Wills Hospital, Philadelphia

JACOB GERSHON-COHEN, M.D.
Assistant Professor in Radiology, Graduate School of Medicine
University of Pennsylvania

EUNICE C. GREENE, M.A.
Department of Biology,
Springfield College

JOHN Q. GRIFFITH, JR., M.D.
John Q. Griffith, Jr., Research Foundation, Philadelphia
Formerly Associate in Medicine, University of Pennsylvania

HARALD G. O. HOLCK, PH.D.
Associate Professor of Pharmacology, Department of Physiology and Pharmacology,
College of Pharmacy, University of Nebraska, Lincoln

HENRY U. HOPKINS, M.D.
Assistant Professor of Clinical Medicine,
University of Pennsylvania

DWIGHT J. INGLE, PH.D.
Senior Research Scientist, Upjohn Research Laboratories,
Kalamazoo, Michigan

WILLIAM A. JEFFERS, M.D.
Assistant Professor of Clinical Medicine,
University of Pennsylvania

CLYDE KEELER, SC.D.
Georgia State College for Women, Milledgeville

JOHN C. KRANTZ, Jr., Ph.D.
Professor of Pharmacology, School of Medicine,
University of Maryland

GEORGE L. KREEZER, Ph.D.
Associate Professor of Psychology,
Washington University, St. Louis

M. AUGUST LINDAUER, M.D.
Instructor in Medicine, School of Medicine,
University of Pennsylvania

THOMAS E. MACHELLA, M.D.
Assistant Professor of Medicine, Associate in Physiology, University of Pennsylvania
Medical School, Chief, Gastro-Intestinal Clinic, University of Pennsylvania Hospital

MAURY MASSLER, M.S., D.D.S.
Associate Professor of Pedodontics, University of Illinois College of Dentistry

RICHARD H. McCOY, Ph.D.
Associate Research Professor of Chemistry,
University of Pittsburgh

JOHN S. NICHOLAS, Ph.D.
Sterling Professor of Biology, Osborn Zoological Laboratory,
Yale University

EUGENE P. PENDERGRASS, M.D.
Professor of Radiology, School of Medicine,
University of Pennsylvania

HERBERT L. RATCLIFFE, Sc.D.
Associate Professor of Comparative Pathology, School of Medicine,
University of Pennsylvania
Pathologist, Zoological Society of Philadelphia

ELLA ROBERTS, M.D.
Associate in Medicine, School of Medicine,
University of Pennsylvania

ISAAC SCHOUR, D.D.S., Ph.D., Sc.D.
Professor of Histology,
University of Illinois College of Dentistry

ALBERT SEGALOFF, M.D.
Director of Endocrine Research, Alton Ochsner Medical Foundation and
Assistant Professor of Clinical Medicine, Tulane University School of Medicine

HARRY SHAY, M.D.
Clinical Professor of Medicine, Temple University School of Medicine
Director, Samuel S. Fels Research Institute, Temple University School of Medicine

REUBEN MYRON STRONG, A.B., Ph.D.
Research Associate in Anatomy, Chicago Natural History Museum; Professor and
Chairman Emeritus, Department of Anatomy, Loyola University School of Medicine

D. H. WENRICH, Ph.D.
Professor of Zoology,
University of Pennsylvania

Dedicated
to the memory of
ALFRED STENGEL, M.D., Sc.D., LL.D.,
President of The Wistar Institute
Vice-President in Charge of Medical Affairs
of the University of Pennsylvania.
This book, a collaboration between
workers in clinical and nonclinical science,
represents an ideal embodied in his own life
as a physician and scientist

Preface to the Second Edition

THE RAT continues to hold its preëminent position as an animal for use in laboratory investigation. While no manual of experimental procedures can satisfy the requirements of all workers, the response to the first edition of this text was enthusiastic enough to require the preparation of a new edition.

The complete text has been reviewed in the light of current knowledge and technics, and necessary revisions made. The appearance of many new reports involving the action and dosage of drugs for rats has necessitated the complete rewriting and considerable expansion of the chapter on pharmacology. New material has been incorporated in the chapters on the digestive system and the osseous system. In many cases it has been a matter of judgment how far technics should be considered when they apply only to specialized fields. The editors hope they have chosen wisely, though naturally their judgment has been influenced in some cases by their own experience and that of their close associates.

The same considerations that recommend the choice of the rat as an experimental animal obtain today as in 1942.

THE AUTHORS.

Preface to the First Edition

THE albino rat has come to be the most widely used laboratory animal. This extensive use is due to a number of factors such as low cost, small space requirement, tractability, omnivorous dietary, short time span of generations, large litters and the fact that the rat can be readily standardized. Moreover the fact that the use of the rat has been and is so widespread enables laboratory workers to compare their results with those of earlier workers in the field and with contemporary research performed in other laboratories.

The preparation of this book has been suggested by the numerous inquiries constantly being received regarding experimental methods applicable to the rat. During the past twenty-five years The Wistar Institute of Anatomy and Biology has maintained a colony of albino rats to provide the necessary material for the investigations carried on in its laboratories. New diets, new cages, and new methods of handling albino rats have been devised. This work has resulted in a "standardized" rat which is an exceptional laboratory animal with characteristics making it suitable for research intended for application to human biology.

The authors have endeavored to prepare a book for all workers employing the rat in laboratory investigation. They have had primarily in mind original investigators but the methods and technics given will prove valuable also for students, rat breeders, and technicians. It should be noted that the technics given, while having particular reference to the rat, are applicable to laboratory work in general.

With certain noteworthy exceptions, the use of small animals in physiologic experimentation has been limited to experiments in which gross changes can be observed or in which a post-mortem pathologic study was the objective. Experiments requiring observations of blood pressure, spinal-fluid pressure, blood volume, and similar procedures, have been limited usually to larger animals such as the dog or cat. Nevertheless, at times there are advantages in the use of smaller animals. As has been pointed out, rats are less expensive than large animals to buy and maintain, their living quarters require less space, and consequently more animals may be used. The animals themselves are easier to handle. Complicated operating boards and holders are unnecessary. Entire organs instead of fragments of organs may be studied normally and pathologically. Moreover, the addition of new species of animals to those already available for experimentation sometimes results in new light being thrown on the problem being investigated.

The present volume is primarily a book of technics and procedures which have been proved satisfactory in our laboratories and in other institutions.

We have developed and revised many procedures in using the rat as an experimental animal. While not all the methods described are original or unique, all have been tested by us and proved satisfactory.

The authors are grateful to Doctors O. H. Perry Pepper, Esmond R. Long, Helen D. King, and J. Harold Austin for advice in the course of the work and the reading of certain manuscripts.

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To Dr. Samuel S. Fels grateful appreciation is due for his personal interest, encouragement, and effort in making the rat a standard laboratory animal.

Thanks are due Mrs. Edmond J. Farris for her drawings and cooperative efforts throughout this book. We also wish to express appreciation to Miss Clara N. Perine, Miss Beatrice E. Roberts and Mr. William Sykes for their kind assistance.

For excellent cooperation, and friendly dealings, the authors wish to thank Mr. E. W. Bacon and the J. B. Lippincott Company.

THE AUTHORS.

Philadelphia

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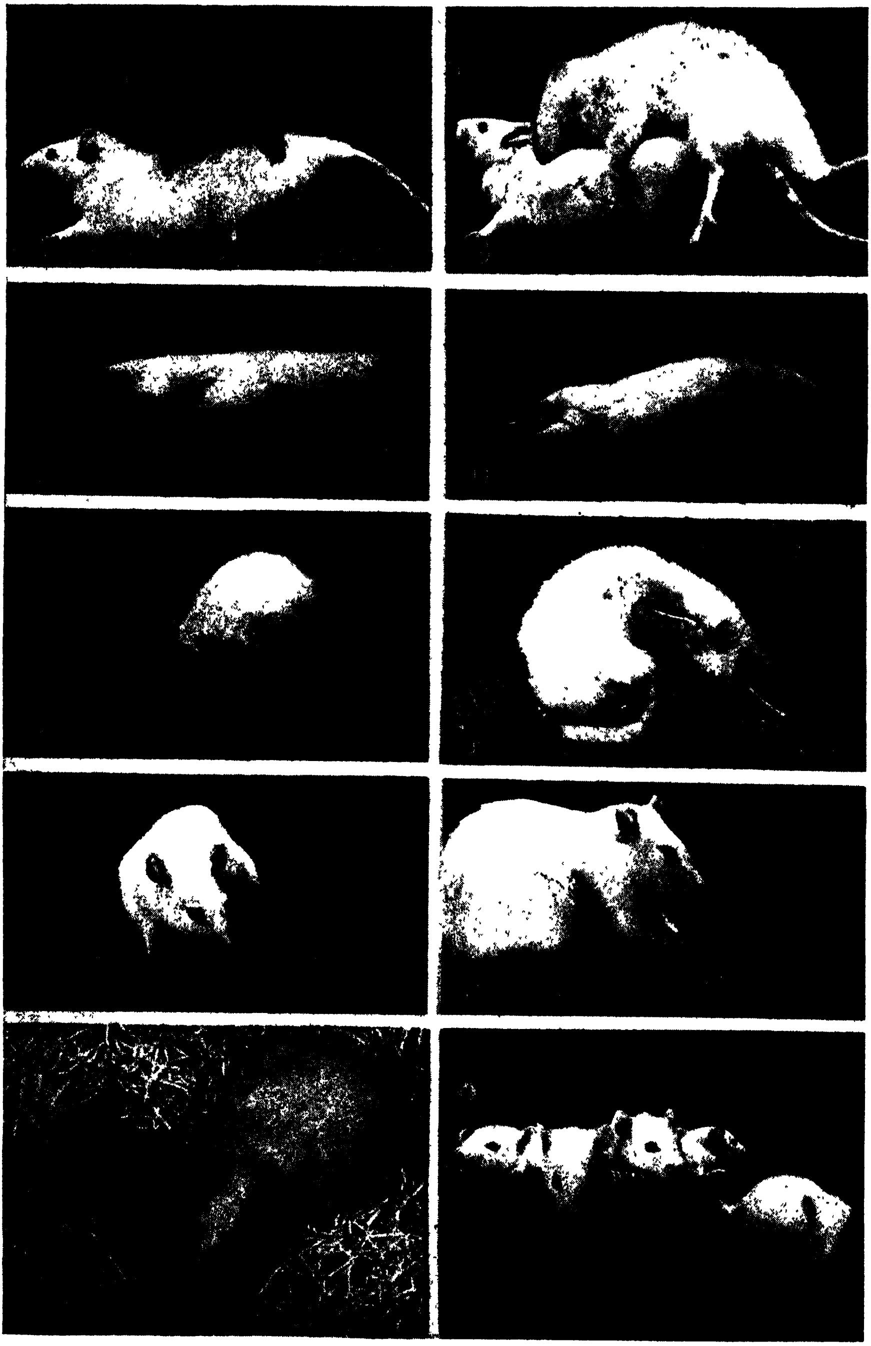
THE RAT IN
LABORATORY INVESTIGATION

Plate II



Breeding and Parturition in the Albino Rat

Estrus: One of the earliest signs of heat in the Wistar strain female albino rat is ear quivering elicited by stroking her gently on the head or back. This reaction does not occur unless the rat is in heat. The copulatory response test by Blandau[20] (pelvic digital stimulation which produces lordosis) follows the ear quivering test, and a positive response (fig. A) assumes that the animal is in heat. When in heat, the female is nervous, apprehensive, darting about, and on touching, braces herself.

The most accurate method (Farris[22]) for determination of onset, regularity and length of heat is by observing the amount of running activity by turntable method (see page 10) during the estrus cycle. Estrus is evidenced by increased running activity which begins at the onset. Other signs of heat are observed by examination of the vaginal smear (see page 52) and in the appearance of the superficial genitalia (dry, with radiating ridges and characteristic blue color rather than the moist, pink condition of diestrum).

Mating: Vigorous Wistar albino males attempt copulation 15 to 70 times in 15 to 20 minutes, with one to two ejaculations, providing the female is in active heat. Note the typical female lordosis during normal copulation (fig. B).

Gestation: In 22 days, 14½ hours (range 10½-21 hours) after mating, parturition begins in the average primipara. A flow is evident usually 1½ to 4 hours previous to first birth.

Parturition Extensions and Contractures: The onset of parturition is characterized by rather regular extensions, with the interval shortening from two minutes to fifteen seconds. During early labor, the female stretches (fig. C) while walking about the cage. With the approach of parturition, the extensions become more severe (fig. D). The female rests on her abdomen, and the hind limbs extend off the cage floor (note left leg). Uterine ripples or contractures are frequently visible on the abdominal wall.

During the extension and contracture period the cervix dilates and the abdominal mass descends. Note abdominal mass before descending (fig. C).

Birth of Young: The first fetus is usually expelled by head presentation, and the others follow either by breech or head delivery. In most cases, the female licks the vulva preceding the delivery. (Failure of the rat to lick the vulva seems to be correlated with difficult delivery.) During delivery she stands in a semi-crouched position (fig. E).

Placenta: The female helps with the expulsion of the placenta by pulling it out with the mouth (fig. F). She promptly eats the placenta (fig. H), holding and working it in her forepaws.

Cleaning Young: The mother licks the young rat and removes the amniotic sheath (fig. G). The mother very seldom pays any attention to the young until the placenta has been eaten.

The Nest: The mother finally assembles the young at the nest site (fig. I). She then cleans herself and permits nursing.

Young: After 21 days the young are weaned (fig. J).

Parturition Time: The length of time for birth of the primipara with litters averaging 11 young is approximately 1½ hours (range 55 minutes through 3 hours, 49 minutes).

Note: Nesting material was removed for this series of pictures, with the exception of figure I.

This study of the mating and birth cycle of the rat was aided by a grant from the Samuel S. Fels Fund. Photographs taken in the Laboratory of The Wistar Institute by Dr. Edmond J. Farris and William Sykes.

1

Breeding of the Rat

By

EDMOND J. FARRIS

THE LONG experience of The Wistar Institute in the breeding and care of albino and other rats for research purposes has brought numerous inquiries concerning the methods used. In this chapter are described briefly the most satisfactory, simple, and efficient procedures to follow in order to raise strong, healthy animals.

BREEDING METHODS USED IN THE WISTAR INSTITUTE ALBINO RAT COLONY

1. Selected breeders are mated for the first time at between 100 and 120 days of age. The males and females used are approximately the same age.

2. To obtain a large number of young, three females and one male are placed together in a cage each Monday morning and the male removed from the cage on the following Saturday morning. A second male is mated with these females on the following Monday, and removed on Saturday.

3. Before the 21st day following the original mating, individual females are placed in separate cages where they remain until their litters have been born and weaned or they have been found to be non-pregnant. Pregnancy is indicated by a marked increase in weight, 25 to 30 grams, and by an enlargement of the abdomen which is evident at about the thirteenth day of gestation.

4. All litters are weaned at 21 days of age. Each member of the litter is then weighed, and those having a weight of less than 30 grams are discarded. (See section on records in this chapter.)

5. Following the weaning of a litter, the mother is given a rest period of about two weeks before being mated again.

6. No stock female is permitted to have more than four litters.

7. Accurate records are kept for each female and male born and maintained in the colony. (See section on records.)

8. A record is made of all males and females that prove to be sterile, and of any abnormalities which may be detected. Unless such animals are being used in special work, they are usually destroyed.

Note: For gray Norway rats and their mutants, it is preferable to breed pairs of litter mates. The female should remain in the same breeding cage until the litter is weaned.

PRACTICAL CONSIDERATIONS

In endeavoring to develop a healthy and uniform colony of rats, important essentials to be considered are:

1. Selection of breeders
2. Intelligent and regular care
3. Temperature of colony room, 72°F with 5° variation, humidity 45-50 per cent.
4. Cleanliness
5. Diet
6. Exercise

These factors will be discussed in the course of the chapter.

Selection of Breeders. Fertility is at its maximum when rats are from 100-300 days of age. At this time the average number of young in a litter should be above the norm (six per litter). As a rule the mothers are able to nurse all young that they may cast.

The proper selection of breeding stock depends primarily upon experience which enables one to recognize the better animals. Breeders should be "streamlined," not plump nor fat. They should be free from all external physical defects, and the coat should have a uniform, smooth and glossy appearance. It is important, also, to select breeders from families that have a record of high fertility.

Doctor Helen King[1] has shown that brothers and sisters may be mated with no detrimental results if breeders are selected carefully. Inbreeding of well-selected breeders tends to eliminate undesirable characteristics, and to produce a homozygous strain which is most desirable for use in various types of experimental work.

Estrus Cycle. Long and Evans[2] studied the estrus cycle in a strain of rats derived from crossing albino females with a wild gray male. They found that the length of the cycle is about four days and that there are five different stages in the cycle (see Chapter 4), each characterized by periodic histological changes in the epithelium of the uterus and the vagina.

It has been shown by Wang[3] that estrus cycle changes and activity cycles parallel each other. In our experience the activity records of individual female rats prove more satisfactory than vaginal smears for determination of the various stages in the cycle, since emotional factors and upsets caused by handling the animals are eliminated.

To secure female albino rats in estrus for experimental purposes, a colony room is darkened completely from 6:00 A.M. through 6:00 P.M. Four 100-watt lights are turned on automatically, and remain on until 6:00 A.M. By this method the rats display more regular estrus cycles, and as a rule come into heat in about ½-3 hours after 6:00 A.M. on the 4th day.

If animal rooms are darkened permanently because of war blackouts, it is advisable to alternate light-dark periods regularly every twelve hours.

Period of Gestation. Careful observations have shown that the gestation period of the albino rat is usually between 21 and 23 days. It may be extended a week or more, however, if a female is carrying a large number of young and suckling a previous litter.[4]

Puberty, Ovulation, Menopause. Both males and females become sexually mature at about 50 to 60 days of age. Long and Evans[2] found that, as a rule, the vagina opens at about 72 days (range 34 through 109 days) and that ovulation begins at about 77 days (range 45 through 147 days). In rather recent observations on 250 male and 262 female Wistar rats Shay (personal communication), reported the vagina open in about 60 per cent of the females between 35-50 days (range 15-67 days). The testes in the males descended in 85 per cent between 18-31 days (range 15-51 days).

Blandau[21] reported ovulation was complete by the 11th hour after the beginning of heat in post-parturitional (and postpartum) rats.

The menopause occurs usually when the females are 15-18 months old.

Parturition. The time required for parturition depends largely upon the age, litter size, and physical condition of the female. It may take but a few minutes, or be prolonged for many hours, the usual litter being born within 1-2 hours. The female eats the placenta which, as Hammett has demonstrated, furnishes a growth-promoting substance to the milk.

Birth Weight. The weight of newborn rats is influenced by several factors: sex, size of the litter, physical condition of the mother and her age. The average birth weight of albino rats in The Wistar Institute colony is about 5.6 grams.

Weight changes according to age of the mother are shown in Table 1.

TABLE 1[6]

Showing the birth weight data for 85 litters of stock and inbred albino rats arranged according to the age of the mothers at the time that litters were cast

	Age of Mother	Number of Litters	Number of Individuals	Males	Females	Total Weight of Males in Grams	Total Weight of Females in Grams	Average Weight of Males in Grams	Average Weight of Females in Grams
1	From 90 to 120 days	27	232	112	120	494.9	494.6	4.41	4.12
2	From 120 to 180 days	36	326	155	171	712.1	736.9	4.59	4.30
3	From 180 to 330 days	17	143	70	73	318.5	321.5	4.55	4.40
4	From 300 to 450 days	5	38	21	17	99.2	73.3	4.71	4.31

Sex Differences at Birth. In newborn rats the sexes can be distinguished by external characters.[7] The male is characterized by a larger genital papilla and a greater distance between the anus and the genital papilla (Fig. 1). Changes in the ano-genital distance at various ages are shown in Table 2.

TABLE 2

Ano-genital distance in young albino rats of various ages

Age	Average Ano-Genital Distance Male	Female
	Mn.	Mn.
Newborn	2 8	1 2
7 days	5 2	2 7
14 days	8 2	4 9
20 days	12 0	7 0
42–50 days	21 0	13 0

Females lack nipples at birth, but show six pairs when they are from 8–15 days old. Occasionally supernumerary nipples are present.

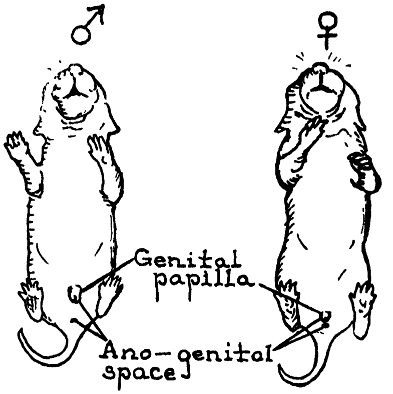


Fig. 1. External sex differences in young rats.

Age Determination. The rat is born hairless, blind, with closed ears, undeveloped limbs and a short tail. Locomotion is effected by wriggling and paddling. According to Donaldson[8] the head is always searching, with quick response to contact of olfactory and taste stimuli. The approximate age of a rat may be determined as follows:

Before birth·
Eyelids of fetus fuse at . the 17th day

After birth:	Days
Ears open at	2½–3½
Incisors erupt at	8 – 10
Young find way to mother	9 – 11
Eyes open at	14 – 17
Hair obscures genitalia	16
Hair obscures nipples in females	16
1st molar erupts	19
2nd molar erupts	21
3rd molar erupts	35
Testes descend	40
Vagina opens	72
The menopause occurs at	450

Life Span. The life span of the albino rat is assumed to be about three years, which may be considered as equal to ninety years of human life.[8]

Under the favorable climatic conditions of California, rats have lived over 40 months.[9] In regions where there is much humidity with high temperature in summer and extreme cold in winter, few individuals live for more than two years.

Domestication. A strain of wild gray Norway rats has been maintained at The Wistar Institute for more than twenty years.[10] These rats are more acutely sensitive than albinos, and are easily startled by any quick, sharp sound or movement and by any new odor, human or otherwise. They can be made as docile as albino rats, however, if they are handled frequently while young.

When tamed, gray Norways can be handled with comparative ease, and thus are excellent for special research work. The strain is homozygous; the animals are large, vigorous, and very prolific, and are more resistant to infection than are other strains of Norway rats.

The breeding procedure outlined for the albino rat is satisfactory for gray rats, but because of their gnawing propensities, gray Norway rats should be kept in metal-lined cages.

Foster Mothers. In certain experimental problems treatment of the mother often causes a lack of milk supply essential for the young. Foster mothers may be substituted with marked success, particularly in the albino strain. King with reference to the wild grays says: "Many females would rear litters from other strains if they were barred from the nest until the young had acquired the nest odor. However, alien young were always killed if the mother had access to them as soon as they had replaced her own offspring."[10]

COLONY BUILDING, CAGES, AND EQUIPMENT

Colony Building. In 1921 The Wistar Institute constructed a colony building which has proven very satisfactory for raising rats. (Fig. 2.) The building contains thirteen well-lighted individual rooms: a record and surgery room, nine rat rooms, kitchen, and cage cleaning room. (Fig. 3.) Each room is maintained in cold weather at a temperature of about 72°, the range being 65 to 75°F. Excessive heat in summer is compensated for by rapid exchange of air in the rooms, which aids also decreasing odors, moisture, and heat generated by the rats themselves. Air conditioning has solved the problems of temperature, humidity, and ventilation in some other colonies. The entire building is screened and protected against entrance of wild rats, cockroaches, and any other animals which may carry infections.

Fig. 2. Animal colony building of The Wistar Institute. An isolated building constructed of brick, concrete, steel, and glass.

The cleaning room is equipped with a tank for washing and sterilizing cages (cage washing machines and automatic bottle washers are available commercially). A large steam sterilizer is located in an auxiliary building. All materials such as bedding, shavings, stored cages, etc., are sterilized before being taken into the colony.

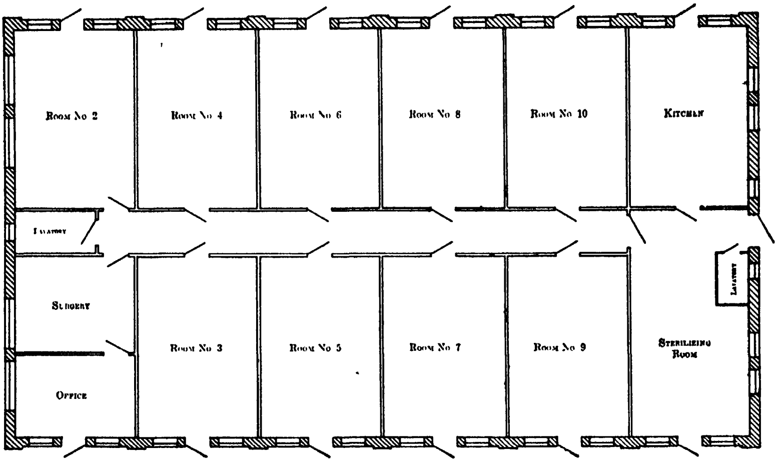
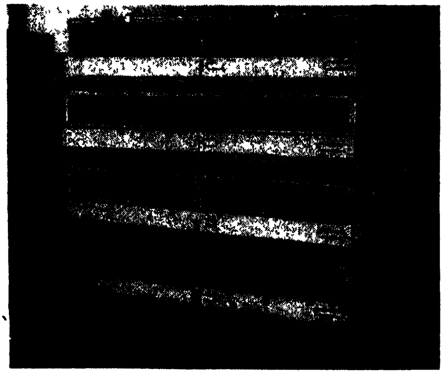


Fig. 3. Floor plan of The Wistar Institute colony building.

The kitchen is equipped with a 50 gallon steam cooker, an auxiliary steam drying apparatus, a power meat grinder and other kitchen equipment.

The colony office serves as a record room where the complete history of each rat may be found. The small room adjoining the office is used for surgery.



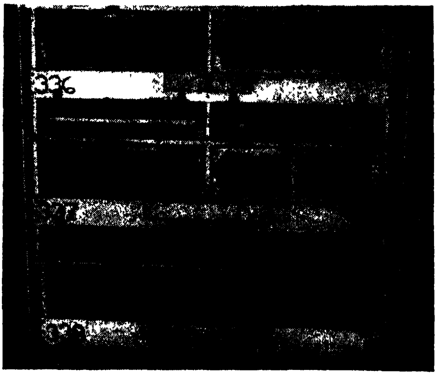


Fig. 4 (*Left*). Type of cage recommended for long-term experiments.
Fig. 5 (*Right*). Dormer type cage.

Cages. After considerable experimentation with various types of cages, The Wistar Institute adopted two forms to satisfy its needs, chiefly for the breeding of large numbers of albino rats and for long-term genetic studies. In considering the selection of cages, suggestions will be offered for cages for short-term experiments based upon a survey trip of rat colonies, both large and small, in the eastern part of the United States.

For long-term experiments the cage shown in Figure 4 is recommended. Cage supports may be placed on casters satisfactorily. Each cage is 10″ high, 10″ deep, and about 57″ long. The end of the cage is all monel metal 10″

by 10″ with a 1″ rim turned on all four edges. The wooden top, back and front of the cage are screwed to the metal ends. The floor of the cage is detachable from the upper portion for cleaning. The entire cage is metal lined when housing wild gray rats. Rats kept in this type of cage have not, as yet, developed ringtail, which is a condition in which new born rats soon develop reddened tails, that become constricted with corrugated rings. The tail, and occasionally toes, blacken and drop off because of this condition.

The dormer cage in Figure 5 is most satisfactory for breeding and housing not more than ten adult rats. The cage is constructed of oak ½″ thick, 35″ long, 12½″ high, 17″ deep, outside measurements. It is divided by a middle partition in which is located an opening, 3″ in diameter, to permit communication between the compartments. The floor of each compartment consists of a movable galvanized wire cloth screen, 15¾″ square (No. 22 wire, ¼″ mesh). This floor is supported along the front and back by monel metal angles. Beneath the removable floor is a monel metal tray, 15¾″ x 15¾″ x ½″ deep, which serves to catch the dirt falling through the cage floor. This cage may be constructed and used without the middle partition.

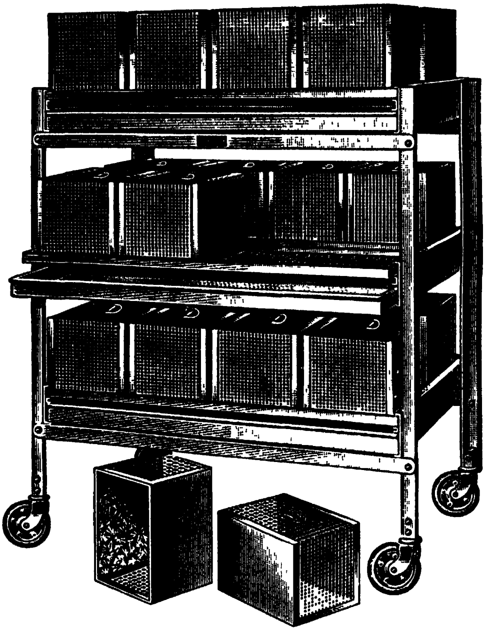
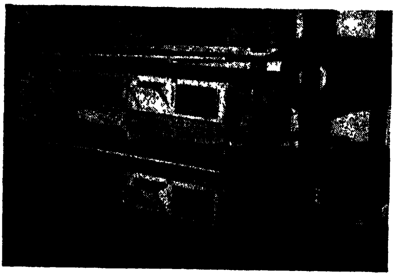


Fig. 6. Movable stand for metal cages.

The wooden cage has been selected in preference to one of metal, for the making is simple and the upkeep less since it does not rust. The cage is odorless, contrary to the usual belief, and may be painted with fluids such as Cresodip (Parke-Davis) which repel lice.

Other laboratories have found that all metal cages are very satisfactory for rats. (Metal cages may be secured from Geo. H. Wahmann Manufacturing Company, Baltimore, Maryland.) They are useful for breeding, weaning, and housing of experimental rats, and are placed in racks or on movable stands mounted on casters. When such stands (Fig. 6) are used, it is customary to place the cages, which have no bottoms, on a wire floor which is mounted above the cage pan. All excreta and food falling through the mesh are collected in the pan below, which is so placed that the rat cannot reach through the mesh or touch any of the material collected in it. The catch pan is made approximately 2″ deep, so that it may be used with nesting material as a suitable floor for cages when they are used for breeding purposes.

By a slight modification of the usual construction, paper may be substituted for the cage pans. Rolls of 40 pound waxed Kraft paper or a similar paper are mounted on the end stand, one roll of paper at each cage level. The paper is passed under the wire floor of the entire row of cage stands. Cleaning is accomplished by withdrawing the soiled paper from beneath the cages at the opposite end of the roll stand. This automatically pulls in clean paper. In a test room in The Wistar Institute this paper system has proven efficient with the dormer cages, as illustrated in Figure 7.



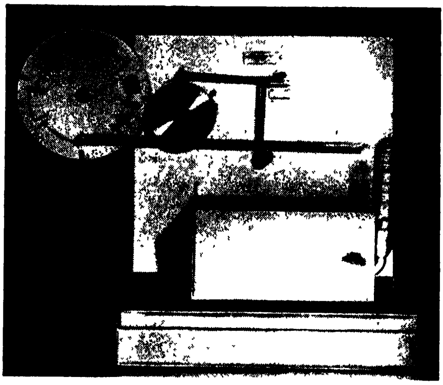


Fig. 7 (*Left*). Rolls of paper for rapid cleaning of cages.
Fig. 8 (*Right*). Back view of exercising cage (Welsh) with recording mechanism.

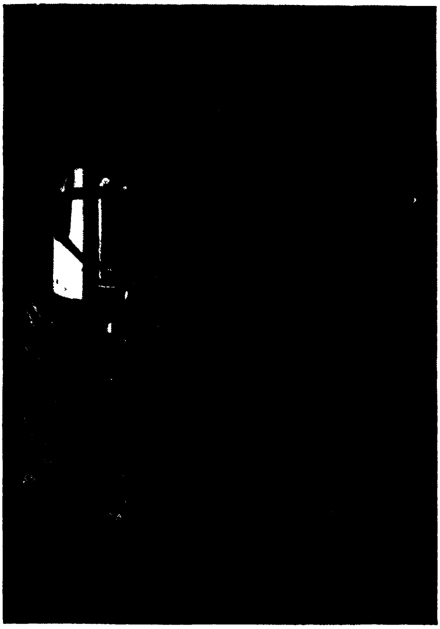
Stands may be constructed to hold 6 rather than 4 individual cages between uprights and in many cases the height increased to accommodate 6 or 8 tiers of cages. Cages close to the floor must be protected from drafts.

Cages of various sizes should be provided to take care of the different needs of the investigators. For keeping one or two animals in a cage, the type shown in Figure 6 is preferred, the rats being readily visible and easily accessible. For breeding and housing individual litters a cage longer and larger is advocated. (Fig. 5.) When fully assembled, one portable stand will hold 24 cages in a space approximately 2 by 3 feet.

Metabolism Cage. A metabolism cage used in many laboratories is shown in Figure 14. This is designed for collection of urine and excreta separately.

Long and Evans[2] describe a clever obstetrical cage designed to record automatically the time of birth of the young. This cage has a ½″ mesh wire sloping floor which permits the newborn young to roll out of the cage and into a box constructed to record the exact time of their exit from the cage.

Exercising Cage. A satisfactory exercising cage for measuring quantitatively the variations in running activity of the rat is shown in Figure 8 (modified after Richter[11]). The cage permits exercise which is essential to the health of the rat. The principle of the recording mechanism is used widely, and has been arranged by Welsh[12] to register automatically the revolutions caused by running activity. (Fig. 9.) The cage consists of small living quarters for the rat with a passage to a running wheel, from the axle of which an eccentric movement operates a cyclometer and finally a writing lever which marks a chart moved by an inexpensive telechron movement timed for one revolution in 24, 48 or 72 hours as indicated in Figure 9.



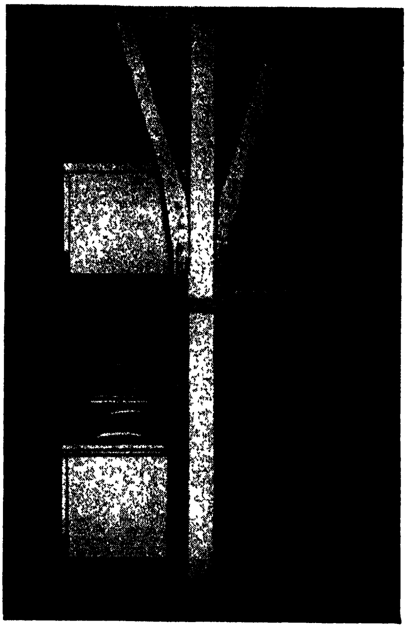
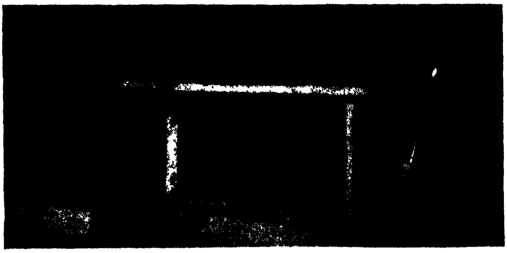


Fig. 9 (*Left*). Side view of exercising cage showing mesh living quarters for rat.
Fig. 10 (*Right*). Exercising drums showing wooden nesting boxes.

It has been observed that rats run farther in large wheels. Therefore, in comparing results it is important that exercisers of the same size be used for all animals in the experiments.

The original apparatus of Richter and Wang,[13] converted by Hemmingsen and Krarup[14] into an automatic register of running activity, later modified by Park and Woods[15] and finally by Welsh,[12] can be improved as follows: The living quarters of the rat should consist of an enclosed box as in Figure 10, rather than wire mesh, so the animal may hide if frightened by unusual noises of the movement of attendants. It has been observed that any disturbance causes the animal to leave the wire mesh living quarters and commence running on the wheel.

Turntable. Instead of using a wheel as exerciser, an excellent yet inexpensive turntable (Fig. 11) may be mounted in the dormer cage. This type of exerciser permits a variety of movements and is easier on the feet of the rat. The turntable (Farris and Engvall[16]) consists of a monel metal disk, 12" in diameter, which can readily be removed from the cage for cleaning. From the brass hub a wire runs directly to a cyclometer. As a rule, the mere entrance of an attendant to read the cyclometer causes the rat to start exercising. To avoid this condition the author has designed an electrical system of recording which may be used with either the wheel or turntable exerciser.



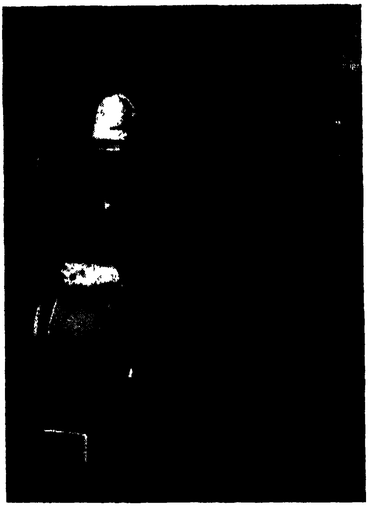
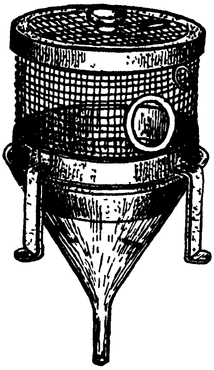


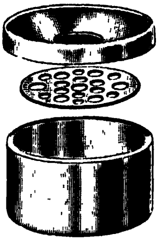
Fig. 11 (*Upper left*). Turntable showing recording attachment.
Fig. 12 (*Lower left*). Water bottle showing brass mouthpiece and food container for dry ration.
Fig. 13 (*Right*). Automatic watering device.

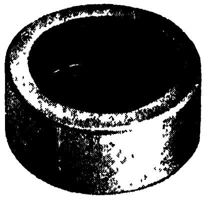
By the use of this device every revolution is recorded by means of a microswitch contact carried to a magnetic Veeder-Root counter. By using this method of recording, the activity of a rat can be followed in another room indefinitely without disturbing the animal. If cages are provided with automatic watering devices and with large food containers a caretaker need not enter the room oftener than once a week.

Tables. Every large colony should be supplied with three substantial tables. One should hold the scales for weighing rats. If the animals are very tame it is feasible to use an automatic scale. (The Toledo scale with platter scoop or pan is recommended.) If the rats are not docile it is more expedient to place an individual in a small wire mesh cage, and to use a counterpoise weight equal to the weight of the cage. An accurate weighing of the rat can then be obtained by using any form of scales having a capacity of at least 1000 grams. When cages are supplied with movable stands, they may be brought to the scales and the rats weighed. If the colony contains only large cages, arranged in tiers and having stationary supports (Fig. 4), the table containing the scales should be mounted on casters. A second movable table is necessary when cages are of the type shown in Figure 4. The caretaker uses this table for containers with food and water supplied daily to the rats. While not a necessity, a table with a drawer is a convenient accessory for any laboratory. It may hold the various small instruments that are used frequently, and serve also for taking notes and recording data.

Drinking Devices. Various drinking devices have been tried in the Wistar rat colony. The most satisfactory one (Fig. 13) consists of an arrangement through which fresh running water is supplied constantly from a reserve tank, drop by drop. It consists of a galvanized cast-iron ring, 2″ in diameter, covered on one end with watertight, transparent, ⅛″ celluloid. An adjustable brass overflow tube is inserted into the lower part of the ring. On the interior of this cast-iron ring a raised edge serves to maintain the water level. The upper part of the ring is bored to receive the water, as shown in the figure.







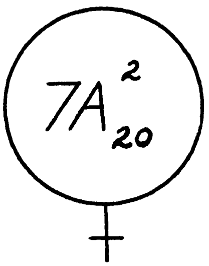


Fig. 14 (*Left*). Metabolism cage.
Fig. 15 (*Upper center*). Food cup—non-scattering type.
Fig. 16 (*Lower center*). Earthen feeder, glazed surface.
Fig. 17 (*Right*). Schematic marking devised by Dr. King.

In case it is necessary to shut off the main water supply from time to time, the device shown in Figure 12 may be used. In this case standard pint or quart bottles serve as reservoirs. Each has a rubber stopper into which is inserted brass tubing, ⅜″ outside diameter with 1/32″ wall and 4″ in length. This is tapped ¼″ deep at one end with a 5/16″ tap, 24 threads to the inch. A plug made from 5/16″ brass rod is screwed into this tapped end, and cut off flush with the end of the tube. A 3/32″ hole is drilled in this closed plug, and finished with a special lathe tool to form a smooth licking surface. The tube is inserted into the rubber stopper 1¼″; the remainder of it is within the bottle to serve as a support, should the bottle be jarred. The tip can be very easily replaced, lasting as a rule about two years even with wild gray rats which constantly gnaw it.

Other drinking devices such as nursery bottles, hair-tonic bottles, test tubes, and glass containers of all sorts have been observed in many colonies. These are usually supplied with tips of bent glass polished only on the licking end and inserted into rubber stoppers attached to the water reservoir.

Feeding Devices. The feeding device shown in Figure 12 has proven very satisfactory for dry ration (pressed biscuit). A ledge on the lower part of the cage catches any smaller particles of food scattered. The animals will eat the food caught on this ledge. For cooked diet a small pan of about 4″ in diameter and 3″ deep is very satisfactory. In quantitative feeding it is important to have a device which prevents food from being scattered yet allows the rat to secure food without great difficulty. Figure 15 shows a satisfactory non-scattering food cup. It is made of monel metal and has a perforated disk which prevents the animal from scattering and digging into the food. An earthenware feeder of the type shown in Figure 16 is used in many other animal colonies, and may serve for a water cup as well. Cold cream and mason jars of various sorts are used in many colonies as food cups.

Bedding. Two chief types of bedding are used in The Wistar Institute colonies. Shavings, pine or poplar, are used in cages with solid bottoms: in dormer cages, with ¼″ screen mesh bottoms, shredded parchment paper is preferred. These materials are absorbent, deodorant, and make excellent nests. Straw, hay, and wood wool or excelsior may be used for bedding, but wood wool is not advised because it gives off a fine dust which is irritating to the rats.

Recorders for Exercising Cages. A counter is necessary to register the number of revolutions made by the exercising drum or turntable. A very satisfactory one to attach directly to the cage is the rotary ratchet counter No. 6. Counters may be secured from the Veeder-Root Company, Hartford, Connecticut. The Veeder-Root magnetic counter is used to record electrically the number of revolutions in another room. By mounting a number of magnetic counters on an individual panel, the cyclometer records can be photographed with accuracy by a time-lapse device.

CARE OF THE COLONY

Washing Cages. The cage-cleaning technique used in the animal colony consists of:

1. Scrubbing cage with soap and water
2. Dipping in hot water
3. Dipping in 2 per cent cresol solution
4. Rinsing with cold water
5. Setting aside to dry

The ordinary commercial dish-washing machine provided with an oversize pump may be used for washing cages. This machine first sprays the cage from top to bottom with very hot soap solution and then with scalding water.

This procedure leaves the cage and parts so hot that they dry almost immediately after being withdrawn from the machine.

Sanitation. The Aeroil Torch (No. 99), giving a temperature of about 2000°F, may be used effectively as a sanitary agent. The lighted torch is moved slowly over the surface of the walls and floor until covered completely. This method is rapid and inexpensive. It is preferable to the high steam method that is used in some colonies.

W. F. Wells[17] has developed a lamp which destroys bacteria by letting down a curtain of ultraviolet rays in the room. It is inexpensive, can be substituted for an ordinary electric light, and may prove very useful in preventing air-borne infection.

PERMANENT RECORDS

Filing. Many methods have been described for filing pedigree records. King's method[18] has proved very satisfactory. The scheme as outlined is as follows: A letter (A or B) places the individual in its proper series The serial letter is preceded by a number which signifies the generation to which the animal belongs. An index number (2, 3, or 4) following the serial letter shows in which of the mother's litters the animal was born; if no index number is used, the rat was a member of its mother's first litter. The subscript after the serial letter is a number which serves to distinguish each particular rat from any other rat belonging to the same generation and litter group. To indicate the sex of the individual, its number is enclosed by the sex symbol. Using King's schematic illustration (Fig. 17), the symbol denotes a female rat belonging in the 7th generation of the strain. She was a member of the second litter cast by her mother, and her individual number in the

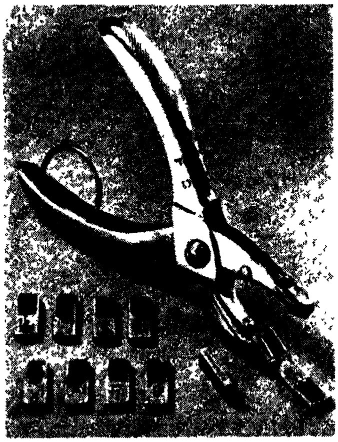
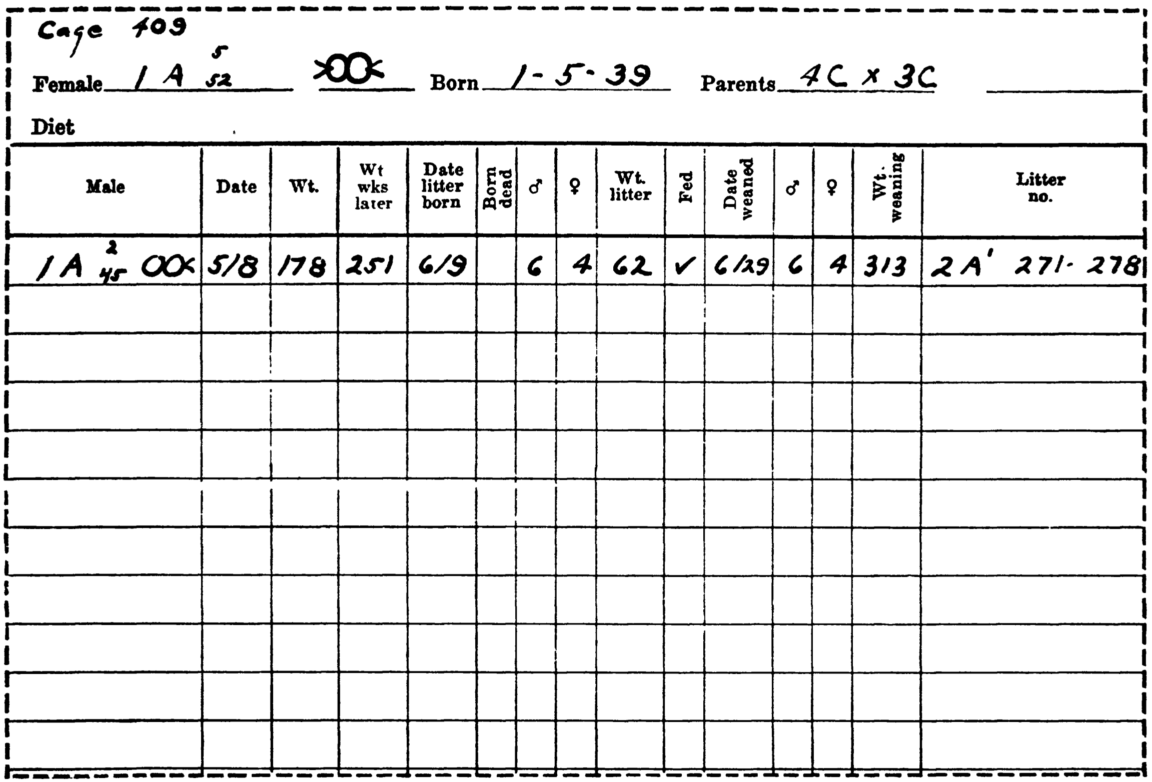


Fig. 18. Punch for tattooing numbers into the rat's ears.



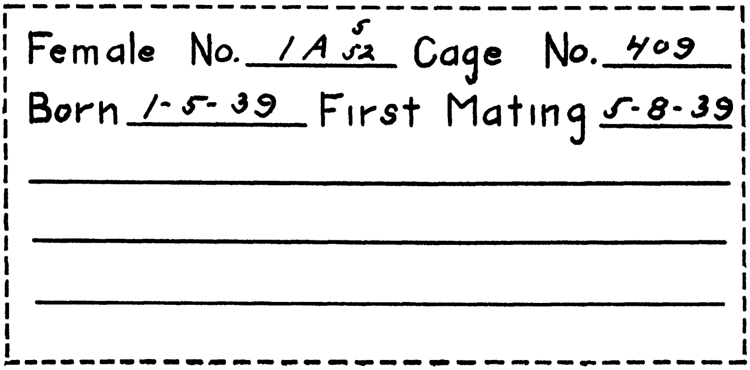
Cage 409

Female 1 A $^{5}_{52}$ OO< Born 1-5-39 Parents 4C x 3C

Diet

Male	Date	Wt.	Wt wks later	Date litter born	Born dead	♂	♀	Wt. litter	Fed	Date weaned	♂	♀	Wt. weaning	Litter no.
1 A $^{2}_{45}$ OO<	5/8	178	251	6/9		6	4	62	✓	6/29	6	4	313	2A' 271-278

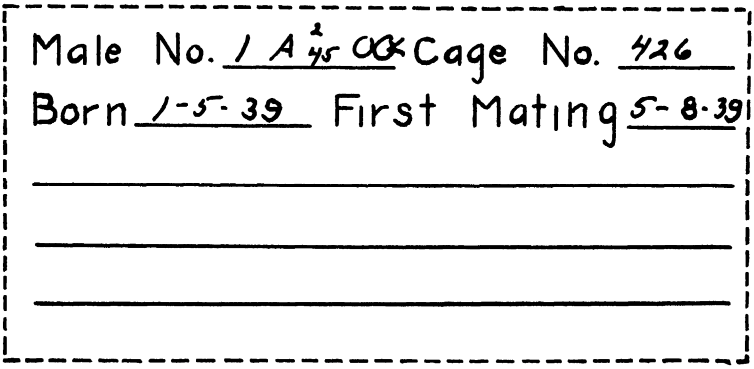
Fig. 19. Female breeder card for breeding room file.



Female No. 1A $\overset{5}{52}$ Cage No. 409

Born 1-5-39 First Mating 5-8-39

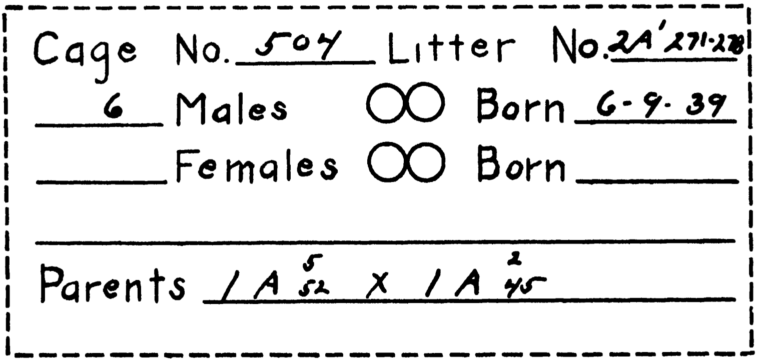
Fig. 20. Card for female breeder.



Male No. 1A $\overset{2}{45}$ OO< Cage No. 426

Born 1-5-39 First Mating 5-8-39

Fig. 21. Card for male breeder.



Cage No. 507 Litter No. 2A' 271-278

6 Males OO Born 6-9-39

____ Females OO Born ____

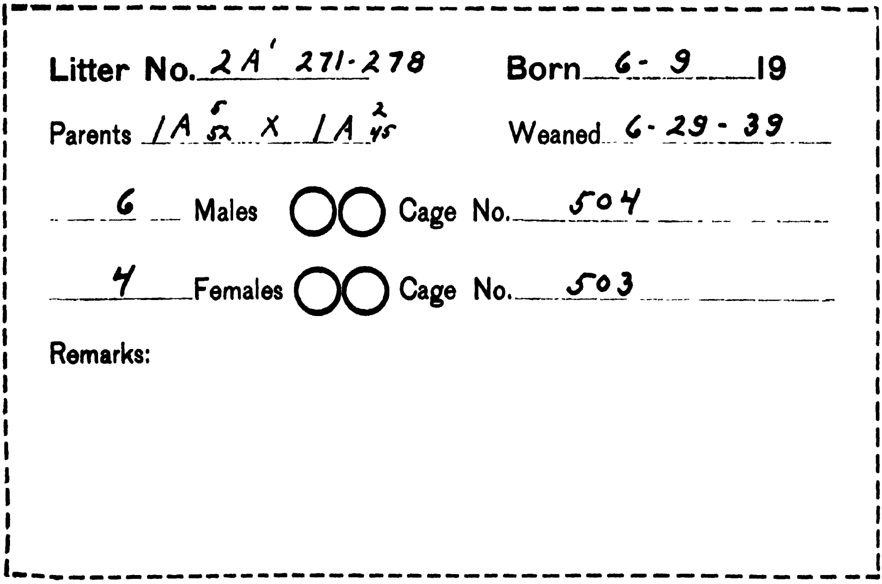
Parents 1A $\overset{5}{52}$ X 1A $\overset{2}{45}$

Fig. 22. Litter card.

series of rats belonging to the second litters of the 7th generation was 20.

Marking. Several satisfactory methods for marking and identifying rats have been developed. One method consists of cutting a small V-shaped wedge into the border of the ear. Two, three and four wedges may be cut in each ear to give a large number of marking combinations.

A new satisfactory punch was developed in The Wistar Institute for tattooing individual numbers in the rat's ears by means of India ink. An ordinary 6-inch ticket punch is used as a holder (Fig. 18). The die on the end of the punch was filed off and replaced with a piece of brass channel ⅜ of an inch long by ⅜ of an inch wide by 3/16 of an inch high. The number blocks are slid into place through the open end. A rubber pad of equal size was cemented to the opposite side. This comprises the holder. Individual digits from "0" to "9" were made by mounting steel pins in the form of the desired number on a piece of medium soft rubber about ⅛ of an inch thick. A small block of brass, approximately ¼ of an inch wide by ¾ of an inch long by 3/16 of an inch high, with a hole drilled through it large enough to accommodate the steel pin number, was placed over the rubber form. The hole containing the number was then filled with hot solder, and the rubber foundation was drawn away from the pins. The pin number block was then filed to fit snugly into the channel holder. Two digits to each ear allow numbering up to 9999.



Litter No. 2A' 271-278 **Born** 6-9 19

Parents 1A $\overset{5}{52}$ X 1A $\overset{2}{45}$ Weaned 6-29-39

6 Males ○○ Cage No. 504

4 Females ○○ Cage No. 503

Remarks:

Fig. 23. Office file card.

Another method is to paint various parts of the rat with a basic carbolfuchsin stain. It is necessary, however, to re-mark the animal about once every six weeks. Another simple method is to perforate the ear with a needle or punch in combinations of one or more dots and then paint the ear promptly with carbolfuchsin or India ink, as described above.

Still another method of marking experimental animals is as follows: the generation is represented by toe amputation, beginning from right to left; the litter number is marked on the right ear, the individual number marked on the left ear.

Recording Data. In the animal colony office is located a perpetual calendar, with individual slots for each day of the year, in which are placed record cards for each new litter. Figure 19 shows one of the 5 various kinds of record cards. Card 1 (Fig. 19) is a female breeder card, and is kept in the breeding room file. Cards 2 and 3 (Figs. 20 and 21) are cage cards, for female and male breeders. Card 4 (Fig. 22) is of a bright color to indicate the presence of a litter. It carries the cage number, date of birth, number of each sex, and mother's individual number, and is moved with the litter at weaning, to serve as a temporary card only. Card 5 (Fig. 23) is filed in the office.

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2

General Methods

By

JOHN Q. GRIFFITH, Jr.

With the Assistance of W. A. Jeffers

HANDLING

Animals which have not been tamed, i.e., are not accustomed to being handled, are best picked up with the palm of the hand over the animal's back, the thumb and index finger folding the forelegs across under the chin (Fig. 24). The animal cannot get his chin down to bite, and the procedure, done correctly, causes little pressure on the trachea. Do not squeeze the body. If the trachea is compressed the animal may have respiratory difficulty later. Workmen's gloves are worn to prevent scratches from the nails of the hind feet. In routine care of the albino rat, it is preferable to avoid the use of gloves. If the rat is difficult to pick up it may be grasped by the tail, whereupon it will pull away and may easily be picked up while straining. The rat cannot be carried around by the tail (as can a mouse) for sooner or later it will climb up its own tail prepared to bite. Forceps may be used in grasping gray rats.

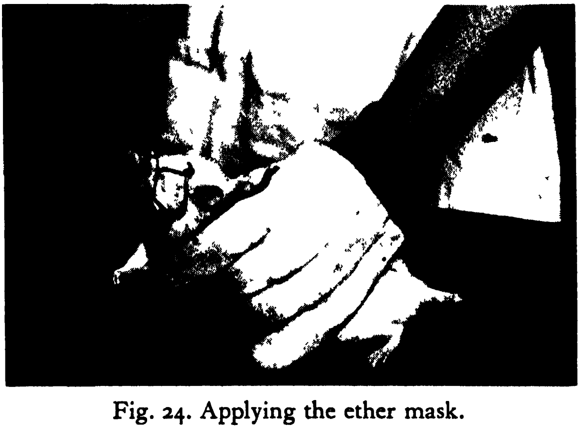


Fig. 24. Applying the ether mask.

METHOD OF SUBCUTANEOUS OR INTRAPERITONEAL INJECTION

Subcutaneous injections can be given most easily into the abdominal wall. With the rat held in the left hand as just described, the needle is inserted with a quick thrusting motion just above Poupart's Ligament, and the point pushed upward about an inch in the direction of the head. One to 2 cc. may then be injected. If larger amounts are to be injected the needle may be partially withdrawn and thrust onward in a slightly different direction. In this way as much as 6-8 cc. may be given through a single needle puncture.

If it is desired that the injection be intraperitoneal, the needle is inserted in the same location and direction but more deeply. The needle should not pass much posterior to the abdominal wall, nor be inserted high enough to damage the liver. If necessary 10-20 cc. of fluid may be given in a single injection.

METHOD OF RESTRAINT WHILE WORKING ON THE TAIL

This is of use in giving intravenous injections into the tail veins, obtaining blood samples, etc. The rat, unanesthetized, is placed on a turkish towel as shown. The towel is folded along the line A-B (Fig. 25), over the rat, and then quickly rolled up and pinned with safety pins, making the pin below the lower legs particularly secure. Rats may be kept thus for hours without harm.

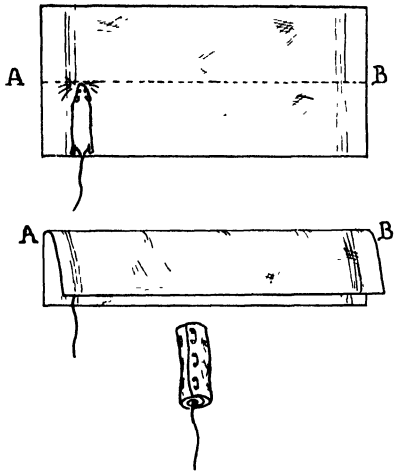
ANESTHESIA

Ether. This is preferable for all operative procedures which are of short duration and in which a mask can be used. A bell jar may be used if desired, the rat being thrust under the jar with a pledget of cotton soaked with ether. This method, however, is wasteful of ether and time-consuming, and after the rat is removed from the bell jar a mask must still be employed. It is simpler to use a mask from the beginning. A very practical one is made of wire covered with gauze. Figure 26 shows the wire in the mask, the adhesive in place, and the gauze added. The gauze is simply wrapped around the narrow end of the mask and held in place by a few stitches.

Holding the rat as described under "Handling," the mask is thrust over its head and held in place. Surgical anesthesia is reached in 2 to 3 minutes. The ears are then pulled in front of the wire, which helps to support the mask. A few drops of ether may be added to the gauze from time to time. This anesthesia is ideal for operative procedures. The chief contraindication is respiratory infection. If such infection is not marked, a preliminary dose of atropine given hypodermically may prevent excessive formation of mucus. The dose for the rat is enormous; ¼ grain atropine sulphate per 100 gm body weight.

Intratracheal Ether Anesthesia. This is of service in experiments in which the chest is to be opened. When combined with curare it may be used to maintain an animal for an hour or more without respiratory excursions.

Under preliminary ether anesthesia by mask the trachea is exposed by a midline cervical incision. Muscles are separated or trimmed away. An opening about 2 mm. in diameter is clipped out of the anterior wall. A glass tube about 5 cm. long, is drawn to capillary size at one end, the outside diameter here not exceeding one millimeter. This is inserted for about 1 cm. into the trachea. It is then connected to the apparatus as shown in Figure 27. All glass tubing used is approximately 4 mm. outside diameter. Rubber tubing is chosen to fit the glass tubing. The size of the bottles shown are only approximate. Raising Tube A so that it approaches the surface of the water decreases the pressure in the tracheal tube, and vice versa. The amount of ether in the gas mixture is increased by lowering Tube B nearer to the ether. We have found, with the set up described, that a water pressure of 10 mm. and Tube B about 40 mm. from the surface of the ether is optimal. Anesthesia may be maintained for one or two hours. It is suitable, however, for acute experiments only, as the tracheotomy wound cannot be closed.



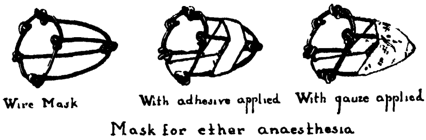


Fig. 25 (*Left*). Wrapping the rat in a towel.
Fig. 26 (*Right*). Steps in making the ether mask.

It is probable that the dose of curare may vary with different preparations and in different animals. In a few experiments we have used 0.1 mg. per 100 gm. of body weight, apparently with success.

Chloroform. We have had bad luck with this anesthetic. Animals may die in a few minutes. After a dozen attempts we abandoned its use.

Nembutal. This is the ideal drug for prolonged anesthesia. We use the powder (Abbott), not the capsules, which vary considerably in strength. We weigh the Nembutal on an analytic balance and make it up in water so that 1 cc. = 6 mg. For deep surgical anesthesia we give 4 to 5 mg. per 100 gm.

body weight; for light anesthesia 3 mg. per 100 gm. is sufficient. If given intraperitoneally anesthesia will be complete in 5 to 15 minutes, and will begin to wear off in 45 minutes. If a longer anesthesia is required a second dose, usually one quarter of the first dose, must be given after 45 minutes. If the injection be given subcutaneously the effect comes on a little more slowly and lasts a little longer. We use the solution only for 48 hours. The water used is distilled but not sterile. Dr. Jeffers gives strychnine when the Nembutal anesthesia becomes too deep. He gives approximately 1/100 grain to a 200 gm. rat. When the Nembutal is accurately weighed, however, this is seldom necessary.

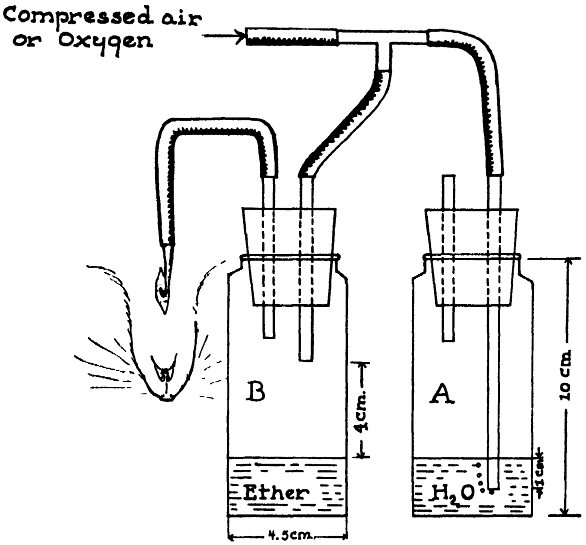


Fig. 27. Diagram of the set-up for intratracheal anesthesia.

Anesthesia with Evipal Sodium. This anesthetic is useful for short operations or for narcosis during the taking of blood pressure readings. Its low toxicity and the rapidity of induction and recovery seem to be indications for its use in animals where respiratory complications would be particularly undesirable.

Since the substance is rather unstable and markedly hygroscopic, it is kept in a dark jar which also contains in a separate container some drying agent. A solution of 10 mg. per cc. of distilled water is made up fresh for each day's work. For intraperitoneal injection the minimal dose per 100 gm. body weight is for narcosis 5 mg.; for anesthesia, 10 mg. The 10 gm. bottles of evipal sodium supplied for rectal use clinically were used. *Caution: Young rats were found to be intolerant of this anesthetic.*

Anesthesia by Freezing. This method was reported by B. P. Wiesner,[1] who used it in the castration of rats 12 hours old. It is only practicable in operations on new-born animals. The purpose is to cool the rat until its body temperature falls to about 2°C, never below 1°C. This may be accomplished by wrapping the rat in filter paper, placing it in test tube and immersing the tube in a thermos bottle filled with ice. The inner wall of the test tube must be dry or the skin of the rat will freeze to it. The rat becomes completely immobile and does not react to stimuli. This condition will last for 3-10 minutes after the rat is returned to room temperature. During this period the operation is performed. The animal is then kept in a cool place for 10-30 minutes, and then gradually warmed, as by holding it in the palm of the closed hand for 20 minutes. After characteristic movements have returned, the animals are cleaned and returned to their mothers. It is advised that at least one young be left in the nest with the mother throughout the operative period, and that the operated animals be returned in front of the nest rather than placed in it. The mother will then retrieve them. After the operated animals have been accepted by the mother, it is advised that, so far as possible, unoperated animals be removed, to eliminate competition by the stronger animals at nursing time. Such precautions are said to lessen the danger of infanticide.

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3

Gross Anatomy

By

EUNICE C. GREENE

This chapter on the gross anatomy of the albino Norway rat is designed to serve as an introduction to the detailed studies which follow; to furnish maps of the areas commonly used for experimentation, and to point out a few of the gross anatomical differences which distinguish the rat from other experimental laboratory animals.

One is constantly impressed with the close structural similarity of the rat to man. In many instances relationships which occur as variations in man are found to be the usual condition in the rat. Several of these differences are contingent upon the difference in posture.

THE SKELETON

Studies of the skeleton have been based upon adult but not senile specimens. In senile material some fusion occurs as in man, although in the long bones there is scarcely a time when the line of diaphysis and epiphysis is not visible.

The **skull** consists of the following bones:

2 Nasals
2 Premaxillaries
2 Maxillaries
2 Zygomas
2 Palatines
1 Vomer
2 Lacrimals
1 Ethmoid
2 Frontals
1 Basisphenoid
1 Presphenoid
2 Parietals
1 Occipital
1 Interparietal
2 Squamosals
2 Periotic capsules
2 Tympanic bullae
6 Auditory ossicles
4 Turbinates
1 Hyoid
2 Mandibles

The vertebral column consists of seven cervical, thirteen thoracic, six lumbar, four sacral, and from twenty-seven to thirty caudal vertebrae. All vertebrae are typical in form except the sixth cervical, which is characterized by a marked peculiarity, a thin plate of bone extending ventrally from the transverse process, the carotid or Chassaignac's tubercle, to which the longus colli muscle is attached.

The thoracic basket is composed of the thirteen thoracic vertebrae, the

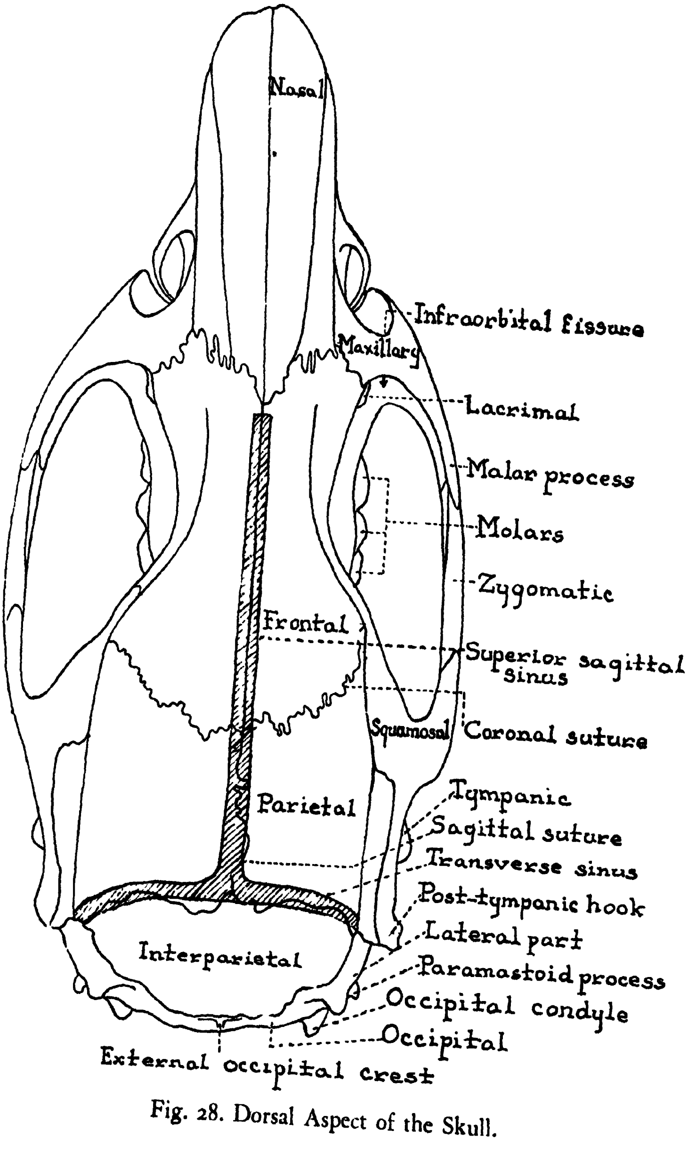

Fig. 28. Dorsal Aspect of the Skull.

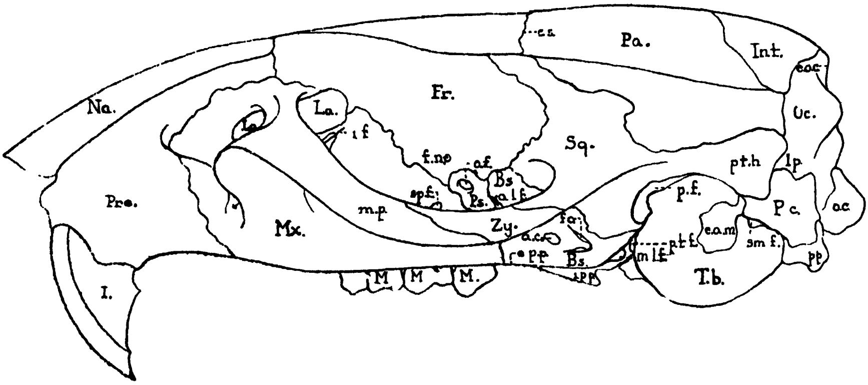

Fig. 29. Lateral Aspect of the Skull.

BONFS

Bs	basisphenoid	Oc	occipital
Fr	frontal	Pa	parietal
I.	incisor	P c.	periotic capsule
Int.	interparietal	Ps	presphenoid
La.	lacrimal	Pre	premaxillary
M.	molar	Sq	squamosal
Mx.	maxillary	T b	tympanic bulla
Na	nasal	Zy.	zygomatic

FEATURES

a c	alisphenoid canal for internal carotid artery.
a l f	anterior lacerated foramen+foramen rotundum for III, IV, VI, and ophthalmic and maxillary divisions of V.
c s.	coronal suture.
e a.m.	external acoustic meatus.
e o c.	external occipital crest.
e p p.	external pterygoid process.
f n.	anterior ethmoidal foramen for nasociliary branch of V.
f.o.	foramen ovale for mandibular division of V
i.f.	infra-orbital fissure for infra-orbital branch of V.
i p p.	internal pterygoid process.
l p.	lateral part of occipital.
m.l f.	middle lacerated foramen for internal maxillary artery.

m p	malar process.
o c	occipital condyle
o f	optic foramen for II
p f	post-glenoid foramen for pterygopalatine branch of internal carotid artery and vein from the lateral sinus
p p	paramastoid process
p t f.	petrotympanic fissure for exit of pterygopalatine branch of internal carotid artery, entrance of chorda tympani, and vein to pterygoid plexus
p t h	post-tympanic hook.
sm f.	stylo-mastoid foramen for exit of VII
sp f.	spheno-palatine foramen for sphenopalatine blood vessels and nerves to the nasal cavity.

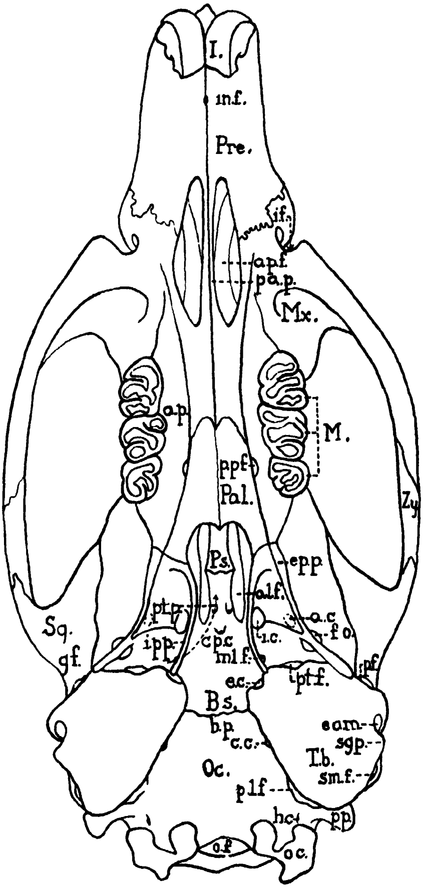

Fig. 30. Basal Aspect of the Skull.

BONES

Bs.	basisphenoid
I	incisor
M	molar
Mx	maxillary
Oc.	occipital
Pal.	palatine
Pre	premaxillary
Ps	presphenoid
Sq	squamosal
T b	tympanic bulla
Zy.	zygomatic

FEATURES

a c.	alisphenoid canal for internal carotid artery.
a l.f.	anterior lacerated foramen+foramen rotundum for III, IV, VI, ophthalmic and maxillary divisions of V, and for ophthalmic vessels.
a p.	alveolar process.
a p.f.	anterior palatine foramen for nasopalatine branch of V to soft palate.
b p.	basilar part of occipital.
c.c.	carotid canal.
c.p.c.	cranio-pharyngeal canal
e a.m.	external acoustic meatus.
e.c.	Eustachian canal.
e.p.p.	external pterygoid process.
f.o.	foramen ovale for mandibular division of V.
g.f.	glenoid fossa for articulation with mandible.
h.c.	hypoglossal canal for XII.
i.c.	interpterygoid foramen.
i.f.	inferior infra-orbital fissure for zygomatic branch of V.
in.f.	incisive foramen for palato-labial artery.
i p.p.	internal pterygoid process.
m l f	middle lacerated foramen for pterygopalatine branch of internal carotid artery.
o c	occipital condyle.
oc f	occipital foramen.
pa p.	palatine process
p f.	post-glenoid foramen for pterygopalatine branch internal carotid artery and vein from the lateral sinus to internal maxillary.
p l f.	posterior lacerated foramen for nerves IX, X, and XI, entrance of pterygopalatine branch of internal carotid artery and int. jug. vein.
p p	paramastoid process.
p p f.	posterior palatine foramen for palatine art. and palatine branch of V.
pt f.	petro-tympanic fissure for exit of pterygopalatine branch of internal carotid artery, entrance of chorda tympani nerve, and vein to the pterygoid plexus.
sm.f.	stylo-mastoid foramen for exit of VII
sg.p.	stylo-glossal process.

ribs, and sternum. The ribs are made up of ventral calcified, and dorsal ossified segments, which, with the sternum, constitute the lateral and ventral elements of the bony thorax. The rat has no true costal cartilages.

The clavicle and its associated elements extend as a chain between the sternum and the scapula in the following order: omosternum, proximal procoracoid piece, clavicle, distal procoracoid piece. Of these the clavicle and the omosternum are ossified, the others are cartilaginous.

The humerus, ulna, and radius present no marked variations from the general mammalian form.

The **carpus** consists of nine bones:

Scapholunar
Triangular
Greater multangular
Lesser multangular
Capitate
Hamate
Centrale
Pisiform Sesamoid
Falciform Sesamoid

The centrale is interposed between the proximal and distal rows. On the ventral surface of the wrist the pisiform has a prominent knob-like projection which helps to form the almost complete circle of bone enclosing the tendons of the wrist. The falciform, extending obliquely across the wrist forms a bar under which the flexor tendons of the digits pass to their insertion.

The pelvic girdle, made up of two ossa coxae, articulates with the first two sacral vertebrae.

On the flexor surface of the distal end of the femur, just proximal to the condyles, are two small but well-defined articular surfaces for the sesamoid bones, the lateral and medial fabellae, which are developed in connection with the tendons of origin of the heads of the triceps surae. The semilunar cartilages of the knee joint are also ossified in the rat.

The fibula articulates proximally with the tibia. Distally they are fused for a short distance even in very young specimens, but the end of the fibula is free and projects slightly beyond the tibia as the large lateral malleolus under which the tendons of the peroneal muscles pass to the foot.

The **tarsus** is composed of eight bones:

Tibiale
Talus
Calcaneus
Navicular
1st Cuneiform
2nd Cuneiform
3rd Cuneiform
Cuboid

MUSCLES

It is assumed that the experimental investigator is concerned with muscles only as they form the landmarks in any operative area, or as they degenerate after nerves or circulation are destroyed. Muscles are therefore labelled in some of the figures shown but for detailed description of origin, insertion, nerve and blood supply, the reader is referred to the author's more extensive work.[1]

VISCERA

In dealing with the viscera, only such points as seem important for investigators, or points wherein the rat differs from other familiar laboratory animals will be emphasized. Among these may be mentioned the lack of tonsils, except as scattered follicular openings, the absence of a gall bladder, the extremely diffuse pancreas, and the presence of a number of glands not recognized in man.

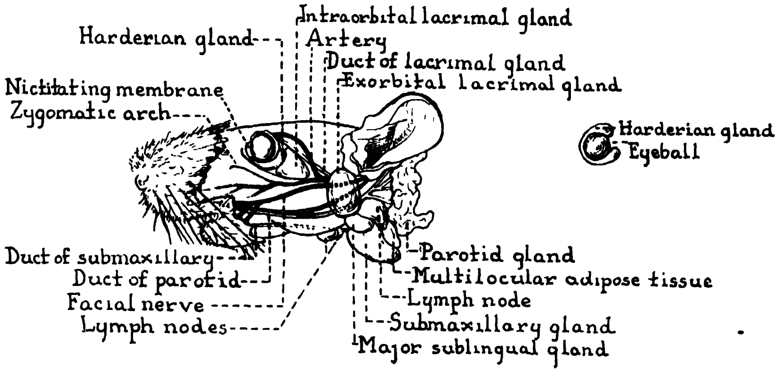

Fig. 31. Neck Viscera, and detail showing relation of Harderian gland to eyeball.

Numerous paired glands of the head and neck (Figs. 31-32) will necessarily be encountered in any dissection of this region. These include both exorbital and intraorbital lacrimal glands; the Harderian gland, its greater portion lying deep within the orbit; the rather diffuse parotid glands; exceedingly large submaxillary glands; and major and minor sublingual glands, the former superficial and closely applied to the latero-anterior surface of the submaxillary gland, the latter lying beneath the mucous membrane of the floor of the mouth, and covering the lateral aspect of the submaxillary and greater sublingual ducts.

Certain large lymph nodes are conspicuous and constant in the head and neck. These will be dealt with later in this volume and need only be mentioned here. They are the submaxillary nodes, practically embedded in the parotid gland and covering the junction of the anterior facial and external jugular veins; and the anterior and posterior cervical nodes (Fig. 33), covered by the omohyoid muscle and lying lateral to the carotid artery and vagus nerve.

The multilocular adipose tissue (Fig. 32) or hibernating gland, so-called, should be mentioned here because it is so extensive and is encountered in dissections of ventral, lateral, and dorsal aspects of the neck, as a diffuse pink structure somewhat fatty in appearance, located chiefly between the scapulae, but sending portions into the neck.

Strictly speaking this structure should not be listed as a gland. A large

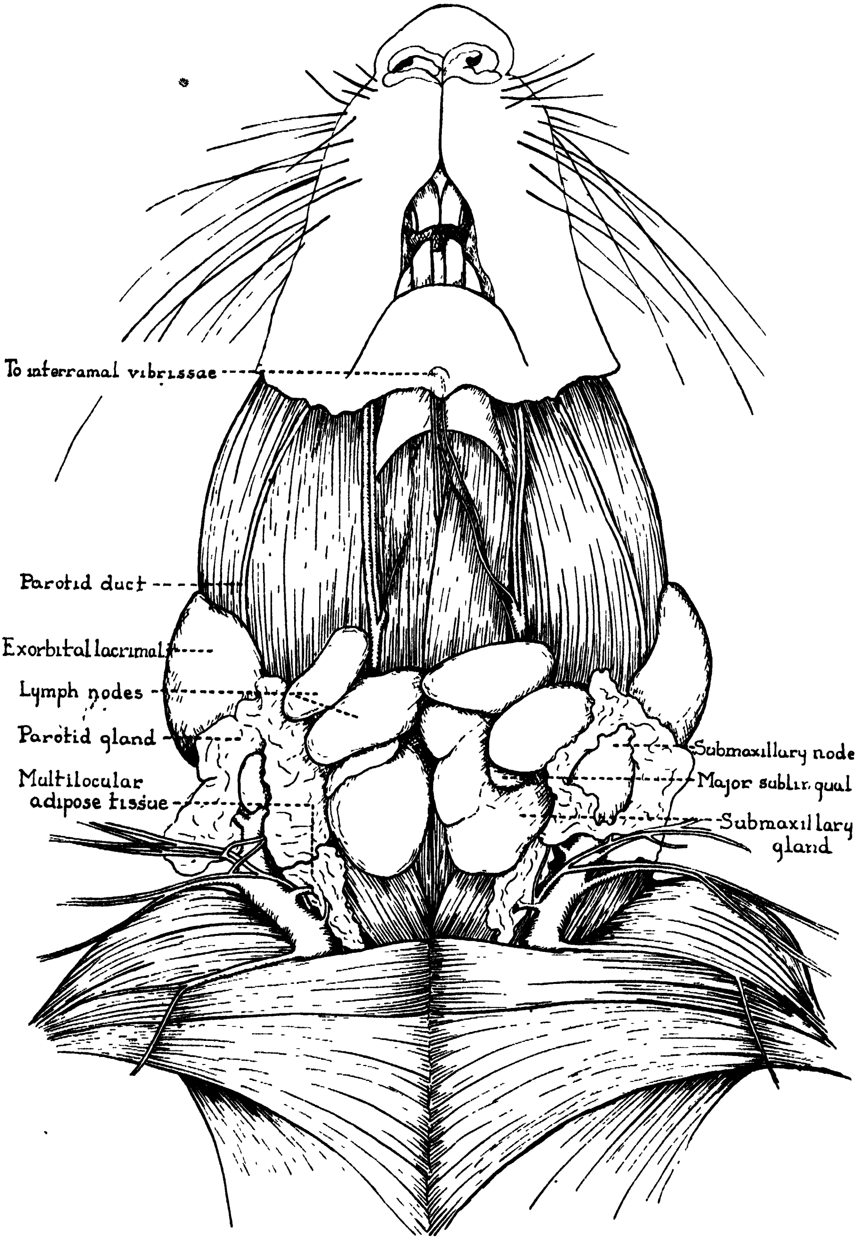

Fig. 32. Superficial Dissection of Neck Viscera.

dorsally toward the shoulder. The abdominal portion spreads out over the lateral wall of the body to become continuous with the relatively thick mass which occupies the inguinal region.

The thoracic viscera (Fig. 34) present no marked peculiarities. An incision in the ventral wall of the thorax reveals the two lobes of the thymus gland, between the two superior venae cavae and covering the trachea.

There are three lobes of the right lung, superior, middle, and inferior, readily visible, and a fourth deeper median lobe which lies in contact with the diaphragm and apex of the heart and is notched to accommodate the inferior vena cava. This is sometimes called the post-caval lobe. The left lung has but one lobe.

Digestive Tract and Associated Glands

The stomach shows an obvious division into two parts, the cardiac portion which is translucent, the pyloric portion which is opaque. Histologically also, these two areas show distinct differences.

The spleen has no gross peculiarities.

The pancreas is a very diffuse low dendritic type with many paired ducts.

The liver presents four parts: a median or cystic lobe, bearing a deep fissure for the hepatic ligament; a right lobe partially divided into anterior and posterior lobules; a large left lobe; and a small caudate lobe which lies deep and fits around the esophagus. Since a gall bladder is absent in the rat, tributary ducts from the various lobes unite to form the bile duct or ductus choledochus.

The intestines show no gross anatomical peculiarities.

The cecum shows a very slight constriction about the middle of the organ which divides it into the large basal portion and a cecal apex. The apex, containing in its wall a mass of lymphoid tissue corresponds to the appendix. The large and small intestines open into the basal portion of the cecum.

Urogenital System

The urogenital system can most easily be described from the dissector's point of view, that is, from the superficial to the deep.

Male. The penis may be extruded from its loose prepuce and shows a single cartilaginous or bony process, the os penis, on its ventral wall.

Lying just under the skin of the prepuce, and opening into its cavity, is a pair of rather slender and flattened preputial glands.

Upon cutting the prepuce and turning the penis upward, its strong suspensory ligament is seen. By separating the bulbo- and ischio-cavernosus muscles at the base of the penis, the pair of small globular bulbourethral, or Cowper's glands, are revealed, opening by slender ducts into the dorsal surface of the urethral flexure.

Within the pelvis, and surrounding the bladder (Fig. 35) are five pairs of organs, relatively much larger than the corresponding structures in man. There are two pairs of prostate glands, one ventral, one dorsal to the ductus deferens; two large hook-shaped and convoluted seminal vesicles, and within the same capsule, along the concavity of the vesicles, are the coagulating glands.

Surrounding the ductus deferens close to its opening into the urethra, is the gland of the ductus deferens.

The testes, which have descended between the 30th and 40th day, lie in two thin-walled scrotal sacs. Outwardly there is no distinct separation of these sacs, but upon removal of the skin the division is seen to be complete. The inguinal canal remains wide throughout life. The internal spermatic vessels become surrounded by masses of fat as they enter the inguinal canal. Careful removal of this fat reveals the testicular artery serving as a core about which is wrapped the pampiniform venous plexus. Within the scrotum several epididymal branches are given off from the internal spermatic.

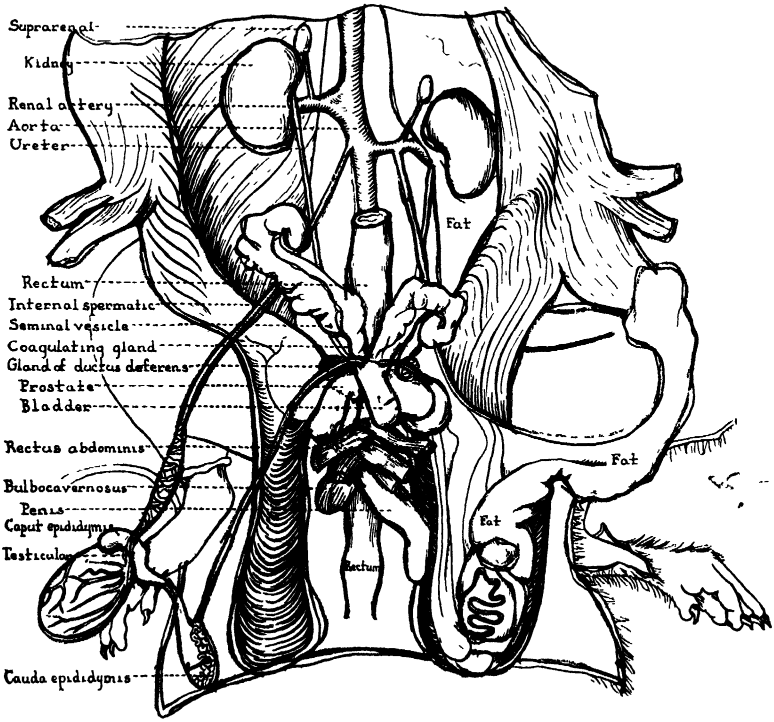

Fig. 35. Male Urogenital System.

The epididymis is a relatively large structure made up of convoluted tubules. It is divisible into three portions: the enlarged caput epididymidis applied to the proximal end of the testis, and almost completely embedded in fat; the corpus epididymidis, a more slender portion, lying along the dorso-medial aspect of the testis; and the cauda epididymidis at the distal pole, which doubles upon itself before giving rise to the ductus deferens.

The ductus deferens, accompanied by the deferential blood vessels, runs proximally through the inguinal canal and crosses the ureter to [illegible]er the urethra.

Female. Anterior to the anus is the vagina, which is closed by a membrane to the time of puberty which is between 70 and 90 days. Anterior to the vagina is the orifice of the urethra at the base of the clitoris, which is enclosed in a small prepuce. Dissection reveals a pair of glands, the bulbi vestibuli just beneath the skin, corresponding to the preputial glands of the male.

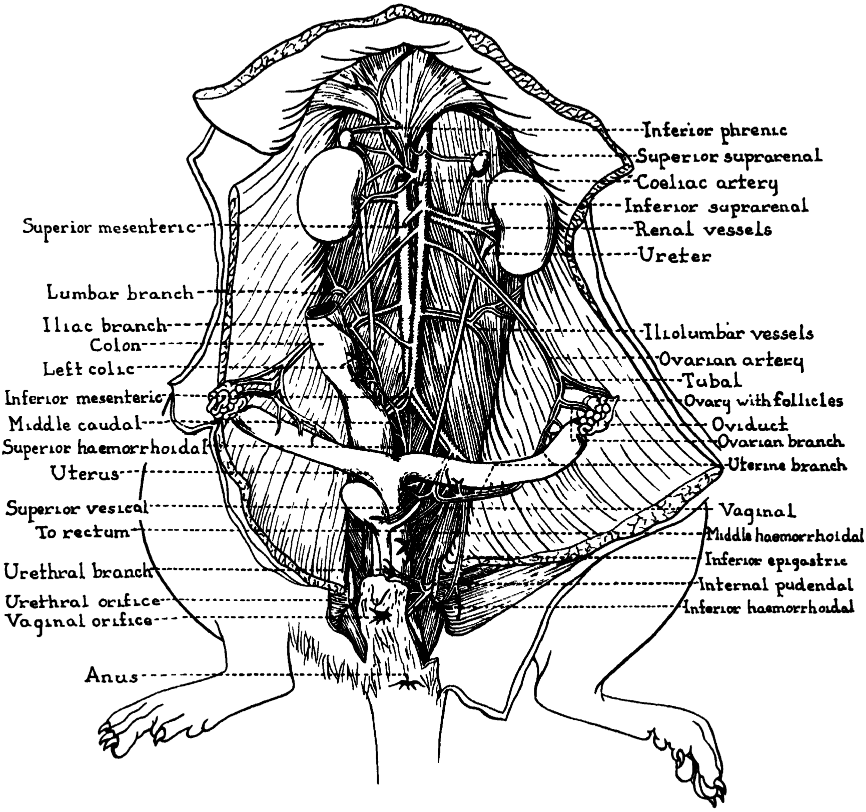

Fig. 36. Female Urogenital System.

Within the abdomen the ovaries are found to lie close to the kidneys along the lateral border of the psoas muscle, embedded in fat. In the adult animal the ovary appears as a mass of follicles with the oviduct a much convoluted tube, its open end applied to the ovary and its distal end opening into the bi-cornate uterus. Though the uterine horns appear to be fused distally there remain two distinct ossa uteri opening into the vagina, as shown histologically.

In the rat the right kidney and suprarenal gland lie at a more anterior or higher level than the left. This is the reverse of the condition found in man. A corresponding difference is noted in the renal vessels. The suprarenals further show a sexual difference, being larger in the adult female than in the male.

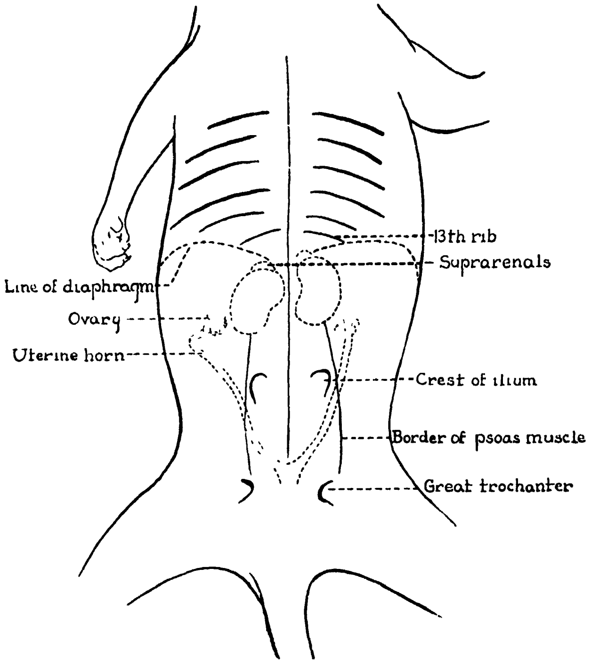

Fig. 37. Dorsal View showing Kidneys, Suprarenals, and Ovaries in relation to the bony landmarks.

ENDOCRINES

In Figure 38 is shown the hypophysis from three aspects. Figures 41 and 42 show its relation to the brain. It is primitive in type with its cavity persisting throughout life.[6] According to Donaldson,[7] a sexual difference in weight of the hypophysis begins to appear at about 40-50 days, increasing with age, the female having the heavier hypophysis.

The thyroid with its attendant parathyroid gland (Fig. 33), lies on the lateral surface of the trachea just below the larynx and covers 4 or 5 tracheal rings. The two thyroids are joined by an isthmus. The parathyroid gland is closely applied to the antero-lateral surface of the thyroid. Accessory parathyroids are rare in the rat.[8]

Other endocrines, the thymus, testis, and ovary, have been described under viscera.

THE NERVOUS SYSTEM

The brain is shown in Figure 39 in relation to the external landmarks of the head. Figures 40-42 show the three usual aspects of the brain and the points of origin of the cranial nerves. Conspicuous characteristics of the rat's brain are the smooth cerebrum, the large olfactory lobes, and the two parafloccular lobes of the cerebellum which lie in deep sockets of the periotic capsule of the skull.

The cranial nerves are typically mammalian in their relationship. In Figure 43 is shown the exit of cranial nerves IX, X, XI, XII and their relationship to the spinal cord.

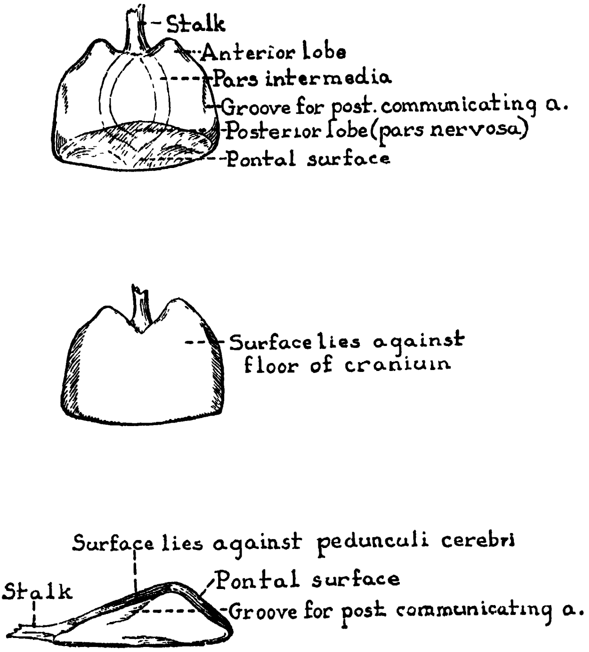
Stalk
Anterior lobe
Pars intermedia
Groove for post. communicating a.
Posterior lobe (pars nervosa)
Pontal surface
Surface lies against floor of cranium
Surface lies against pedunculi cerebri
Stalk
Pontal surface
Groove for post. communicating a.

Fig. 38. The Hypophysis.

The spinal cord showing typical cervical and lumbar enlargements, ends at the level of the fourth lumbar vertebra, with the filum terminale traceable into the tail beyond the third caudal nerves, while the cauda equina, which is made up of all the lumbar, sacral, and caudal nerves, conceals the extent of the cord itself.

The spinal nerves consist of 34 pairs; 8 cervical, 13 thoracic, 6 lumbar, 4 sacral, and 3 caudal. The first cervical nerves lack a ganglion. The ganglion of the second cervical is exposed in the angle between the atlas and epistropheus. All other spinal ganglia are enclosed within the vertebral canal. From the 2nd or 3rd thoracic to the 1st sacral nerves, pairs of rami communicantes connect the spinal nerves with the sympathetic ganglia. In the lumbar region the rami are long and slightly irregular in their distribution.

The cervical, brachial, and lumbosacral plexuses are fairly typical though not divisible into cords as in man.

THE SYMPATHETIC SYSTEM

The sympathetic system consists of two trunks or chains with 24 pairs of ganglia; 3 cervical, 10 thoracic, 6 lumbar, 4 sacral, and 1 caudal.

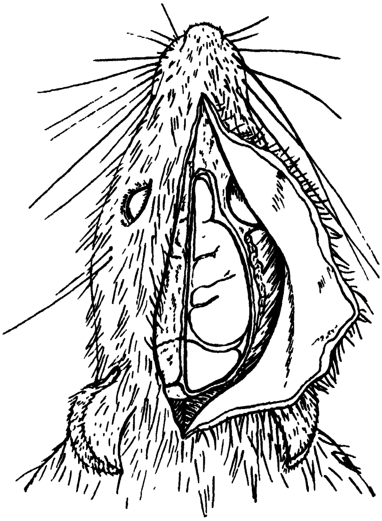

Fig. 39. Dorsal Aspect of Head showing Brain in relation to the exterior.

Emerging from the carotid canals of the skull, the two trunks can be traced into the tail where they become lost as minute filaments along the caudal artery.

The cervical sympathetic ganglia (Fig. 43) are superior, middle, and inferior. As the sympathetic trunk emerges from the skull it accompanies the internal carotid artery and its first, or superior cervical ganglion, is found at the branching of the common carotid into external and internal carotid arteries.

Lying dorsal to the common carotid artery and the vagus nerve, and running close to the bodies of the cervical vertebrae, the trunk reaches the level of the first rib where it bears a middle cervical ganglion, and between the 2nd and 3rd ribs, the third or inferior cervical ganglion. The subclavian loop (ansa subclavia) crossing the subclavian artery ventrally, unites these two ganglia.

The middle cervical ganglion is commonly creased by a long groove which accommodates the beginning of the vertebral artery.

Gray rami communicantes from the middle cervical ganglion connect with the fifth, sixth, seventh, and eighth cervical nerves and, together with the vertebral artery, enter the vertebrarterial canal to form the vertebral plexus.

The inferoir cervical ganglion sends a cardiac branch to the heart.

Typical segmental pairs of gray and white rami communicantes are found

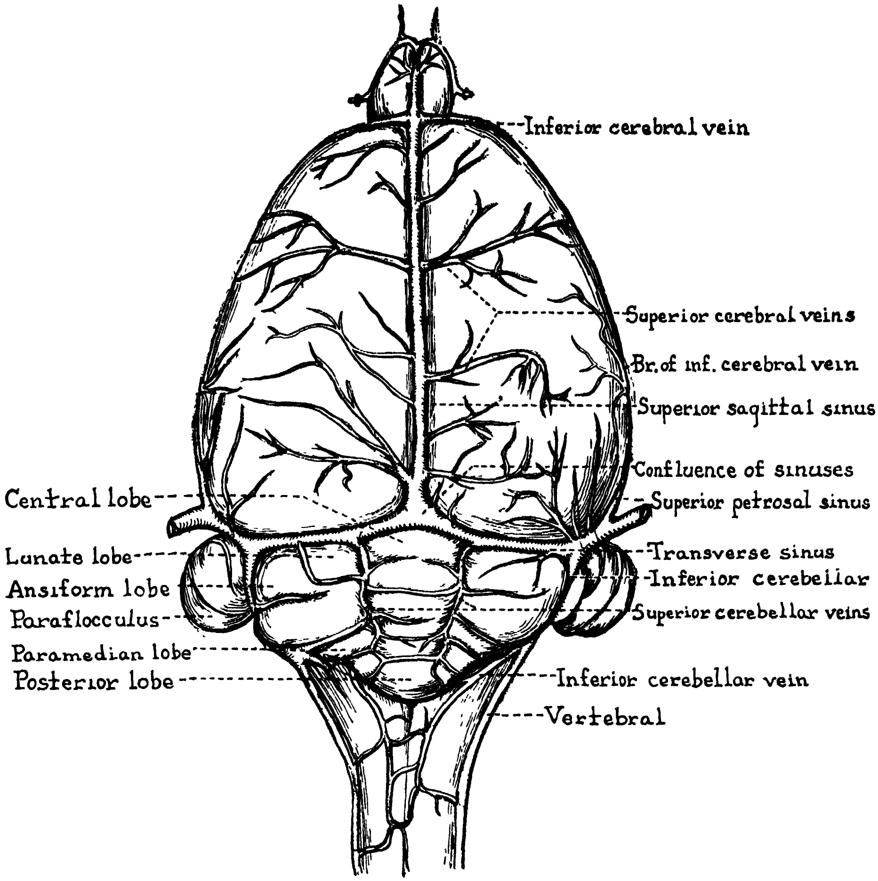

Fig. 40. Dorsal Aspect of the Brain with its Superficial Vessels.

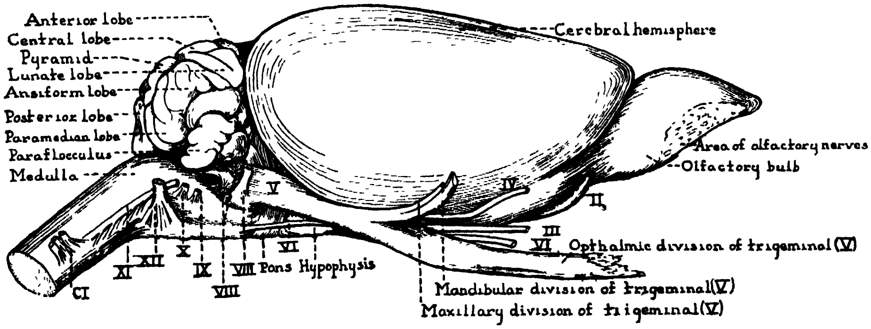

Fig. 41. Lateral Aspect of the Brain and Roots of the Cranial Nerves.

connecting the sympathetic or spinal ganglia with the anterior rami of the corresponding spinal nerves from the fourth thoracic to the first sacral.

The greater splanchnic nerves are made up of contributions from the last four thoracic and first lumbar sympathetic ganglia. They pierce the diaphragm to connect with the coeliac ganglion lying on the ventral surface of the aorta between the coeliac and superior mesenteric arteries. From the coeliac ganglion fine plexuses are distributed to the diaphragm, along the superior mesenteric artery, and to the adjacent viscera.

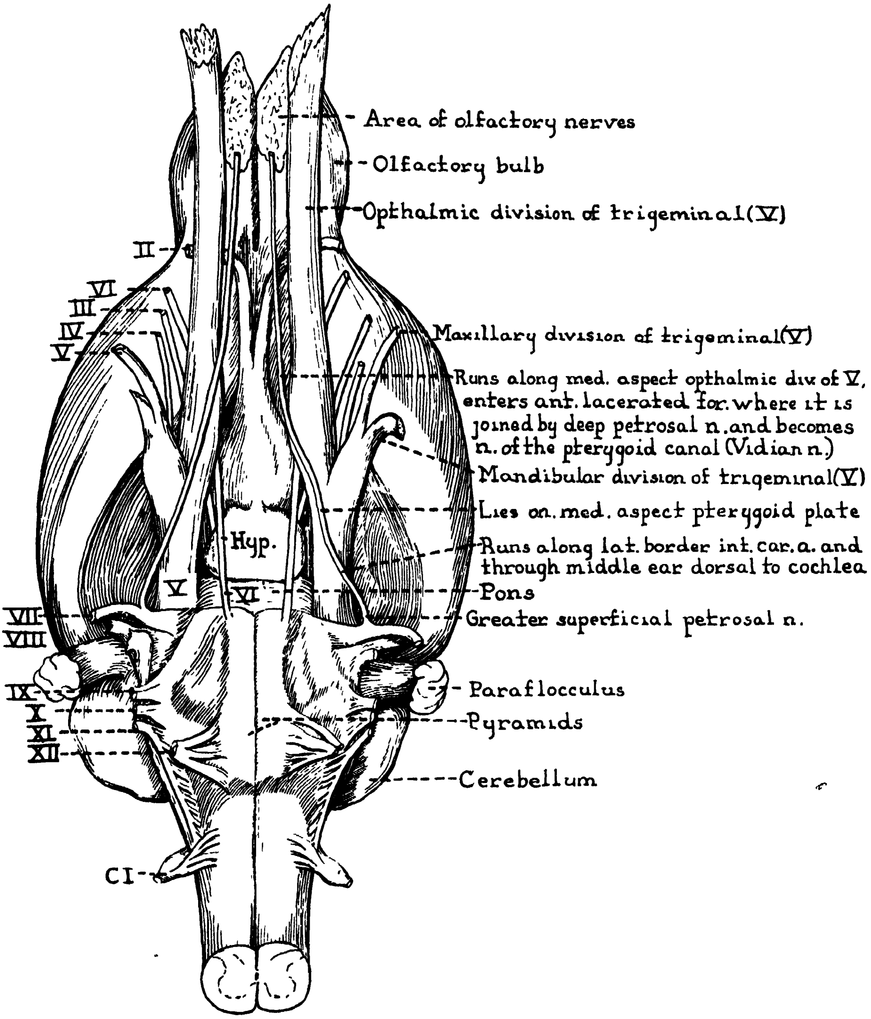
Area of olfactory nerves
Olfactory bulb
Opthalmic division of trigeminal(V)
II
VI
III
IV
V
Maxillary division of trigeminal(V)
Runs along med. aspect opthalmic div. of V, enters ant. lacerated for. where it is joined by deep petrosal n. and becomes n. of the pterygoid canal (Vidian n.)
Mandibular division of trigeminal(V)
Lies on. med. aspect pterygoid plate
Hyp.
Runs along lat. border int. car. a. and through middle ear dorsal to cochlea
V
VI
Pons
VII
VIII
Greater superficial petrosal n.
Paraflocculus
IX
X
XI
XII
Pyramids
Cerebellum
CI

Fig. 42. Ventral Aspect of the Brain and Roots of the Cranial Nerves.

The lesser (small) splanchnic nerve from the level of the third lumbar ganglion also joins the coeliac plexus.

The least (lowest) splanchnic nerve arises from the third or fourth lumbar ganglion and accompanies the abdominal aorta from the level of the renal artery, lying at first dorsal, then lateral, then ventral to the aorta and finally uniting with the nerve of the opposite side in a ganglion which lies at the bifurcation of the aorta. From this ganglion a slender pair of nerves runs with the inferior mesenteric artery to form the inferior mesenteric plexus supplying the pelvic viscera.

CIRCULATORY SYSTEM

No attempt can, or need be made in this limited space to describe the complete circulatory system. A few rather comprehensive figures of the principal vessels are shown, especially in the areas commonly involved in experimental work, and certain peculiarities of the rat correlated with its quadrupedal position, will be brought out.

Branches of the external carotid artery are shown in Figure 44. The internal carotid artery is a relatively more important artery in the rat than in man since it has taken over the pterygopalatine artery which in man arises from the internal maxillary branch of the external carotid. This artery enters the posterior lacerated foramen of the skull with the internal jugular vein, crosses the tympanic bulla to emerge with the chorda tympani through the petrotympanic fissure and divide into pterygoid and palatine portions.

The circle of Willis has been variously interpreted by different investigators. Adachi,[9, 10] who has made one of the most exhaustive studies of the variations of the circle in man, says that, "by the arteria communicans posterior is understood the connection between the internal carotid and the basilar arteries, so that the posterior cerebral does not in the ordinary sense, spring from the basilar but from the posterior communicating artery." The anterior, carotid part, of the posterior communicating may be stronger or the posterior basilar part may show the greater caliber. Adachi shows all types of variation within this generalized scheme and there is every evidence to indicate that we may expect to find just as extensive variation in the rat as Adachi finds in man.

Of the veins of the head, the transverse or lateral sinus (Fig. 40) is the most peculiar, not in its origin or branches but in its course. Extending laterally along the attachment of the tentorium, the transverse sinus makes its exit from the skull through the postglenoid foramen directly behind the condyle of the mandible (Fig. 45), to unite with the internal maxillary vein before the latter joins the superficial temporal, or it may be a tributary of the anastomotic vein between the internal maxillary and inferior alveolar veins. A similar condition occurs in rare instances in man. Figure 46 shows the superior sagittal, or longitudinal, and transverse sinuses in relation to the bones of the skull.

The three jugular veins (Fig. 44) in the rat differ markedly in relative size from those in man. The external jugular vein is the principal vein of the neck in the rat, being exceedingly large, superficial and conspicuous in any dissection. Made up of posterior and anterior facial veins it runs posteriorly along

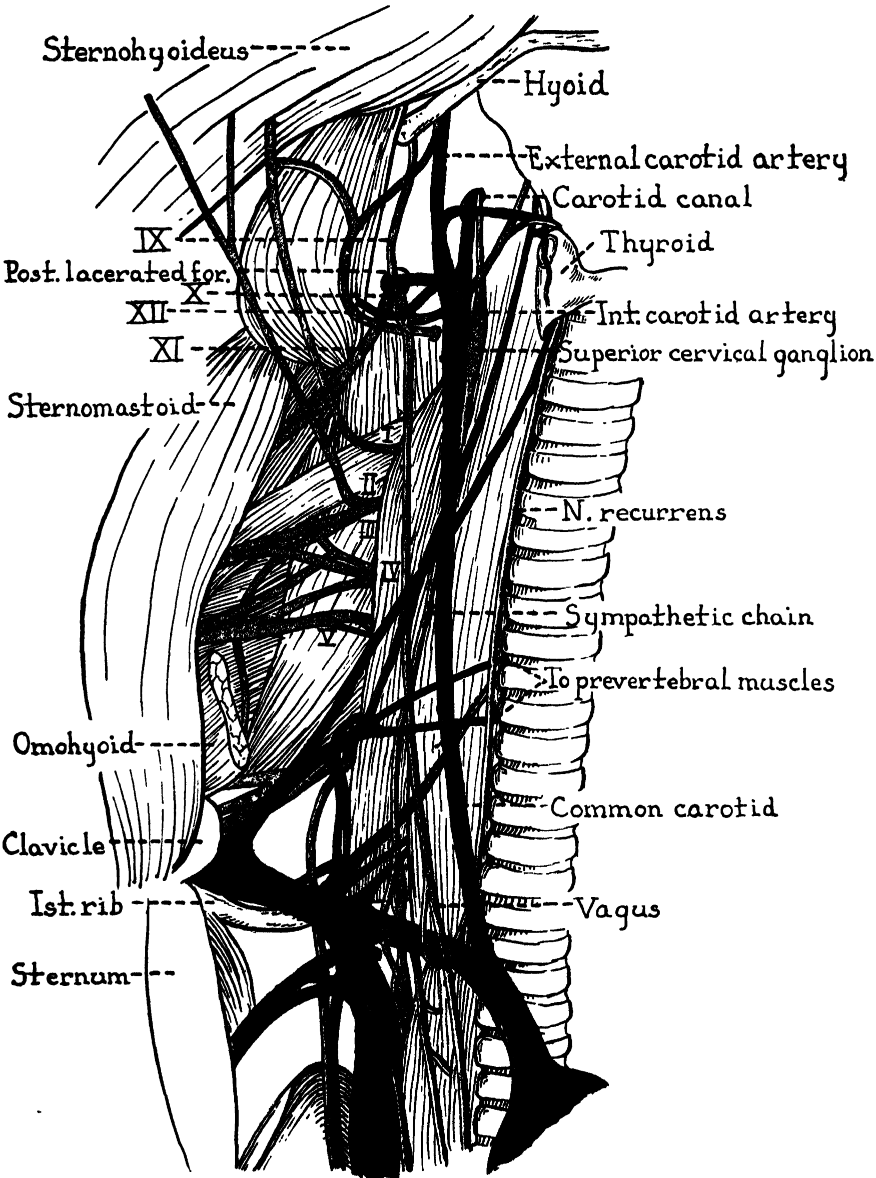

Fig. 43. Cranial Nerves IX, X, XI, XII, and Sympathetic Trunk emerging from the Skull.

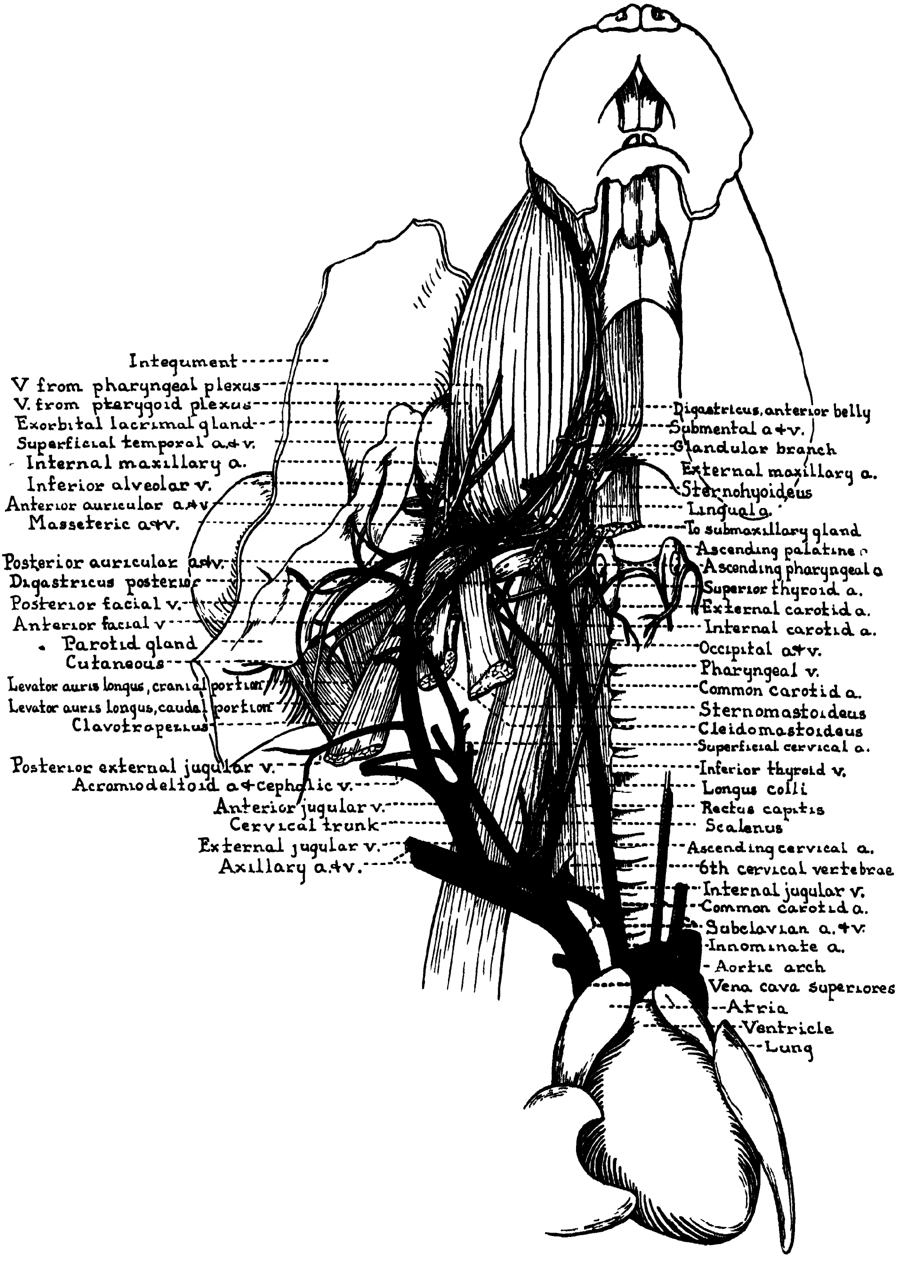

Fig. 44. Branches of the External Carotid Artery and Tributaries of the External and Internal Jugular Veins.

the boundary between the sternomastoid and clavotrapezius muscles, crosses the middle of the clavicle superficially and unites with the axillary vein to form the subclavian.

The anterior jugular vein, lying deep to the clavicle, unites generally with the subclavian vein though it may frequently enter the external jugular before the latter joins the axillary.

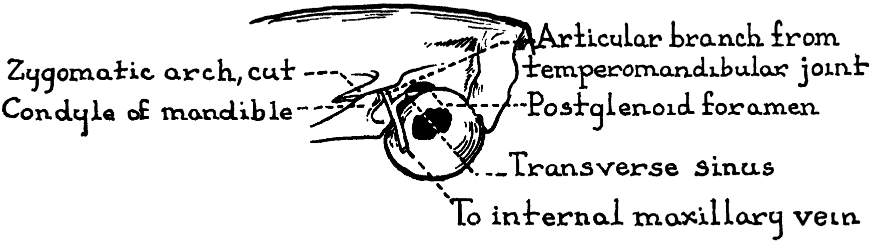

Fig. 45. Detail showing Transverse Sinus leaving Cranium.

The internal jugular vein, relatively much larger in man, is small in the rat because it has no communication with the anterior and posterior facial veins. It is made up of tributaries from the occipital and inferior petrosal sinuses, leaves the skull through the posterior lacerated foramen, takes a deep course through the neck, and finally crosses ventral to the carotid artery to unite with the subclavian vein to form the vena cava superior.

The thoracic lymph duct opens into the left subclavian at its junction with the internal jugular. This is associated with the persistence of the left vena cava superior in the rat.

Vessels of the Upper Extremity. The subclavian artery (Fig. 47), from the innominate on the right, and from the aortic arch on the left, runs between the first rib and the clavicle to leave the thorax and become the axillary artery.

The axillary artery is that portion of the arterial trunk from the first rib to the point where it leaves the axilla between the latissimus dorsi and cutaneous maximus muscles, from which point it becomes the brachial artery.

The brachial artery continues through the brachium to the elbow joint where it passes into the antibrachium and divides into ulnar and median arteries. The radial artery leaves the brachial just above the bend of the elbow.

Most of the branches of these vessels show the typical mammalian pattern. A few points characteristic of the rat should perhaps be mentioned.

The cervical trunk corresponds, in the main, to the thyrocervical trunk of man but lacks the thyroid element which has been taken over by the deep cervical.

The circumflexo-subscapular trunk is the name given to the short thick trunk from which spring several branches which in man generally arise independently from the axillary, namely, anterior and posterior humeral circumflex, and the subscapular arteries.

The cephalic vein is superficial and conspicuous. Curving around the radial surface of the antibrachium to the cubital region, it runs upward along

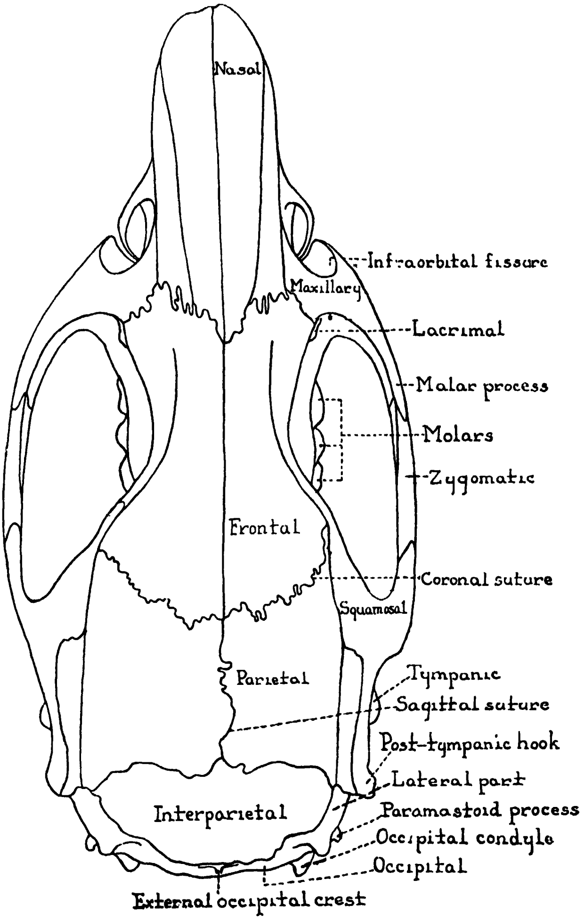

Fig. 46. Superior Sagittal and Transverse Sinuses in relation to the bones of the Skull.

the biceps to the boundary between pectoralis and acromiodeltoid muscles and follows this boundary to reach the external jugular vein at the level of the clavicle.

The axillary vein with the corresponding artery extends from the point where it enters the axilla between latissimus dorsi and cutaneus maximus to the first rib where it joins the external jugular and enters the thorax as the subclavian vein. Here it immediately unites with the internal jugular vein to form the vena cava superior.

With the persistence of two venae cavae superiores in the rat, there is a fairly symmetrical arrangement of the tributaries of these veins.

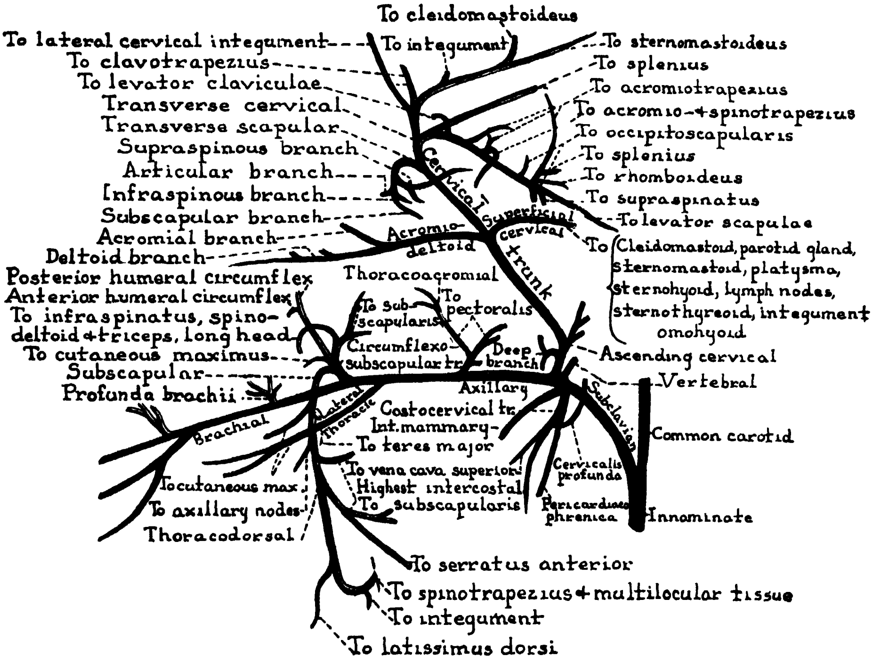

Fig. 47. Schema of Branches of the Subclavian Artery.

The azygos vein, which in man lies to the right of the aorta, lies on the left in the rat, obviously due to the persistence of the left vena cava superior into which it opens.

Branches of the Aorta. The thoracic branches of the aorta and their corresponding veins present no important peculiarities. Certain visceral branches of the abdominal aorta show variations which should be mentioned here.

Of the renal arteries, the right arises somewhat higher than the left, corresponding to the higher kidney, and is frequently found above the superior mesenteric. Both renal arteries give rise to the inferior suprarenal branches (the superior suprarenals being branches of the inferior phrenics), and may give rise to the internal spermatics in the male. Collateral circulation from the inferior phrenic to the cortex of the kidney may occur, making it difficult to eliminate all circulation to the kidney, by ligating the renal vessels:

The renal veins, like the arteries, differ from those in man. The left renal opens into the vena cava inferior well below the right. The inferior suprarenal veins open into the renals.

The internal spermatic arteries of the male usually arise from the aorta below the renals, the right higher than the left, but may be branches of the renals.

The ovarian arteries of the female arise from the aorta, the right higher than the left.

The left testicular (internal spermatic) vein of the male and the left ovarian vein of the female enter the left renal vein, while the right testicular and the right ovarian enter the vena cava inferior.

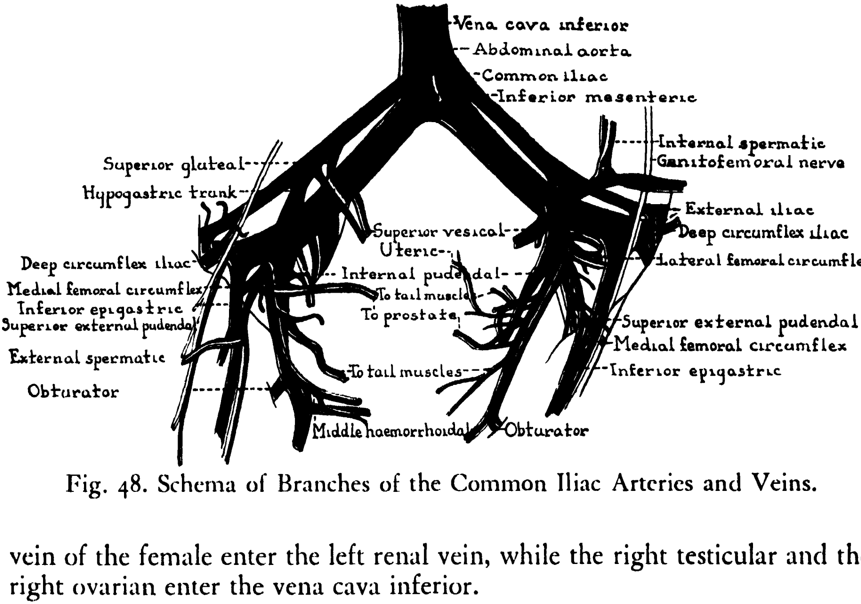

Fig. 48. Schema of Branches of the Common Iliac Arteries and Veins.

The Portal Veins. The lienal (splenic), superior mesenteric, and the pyloric veins make up the portal system. Collecting tributaries from the abdominal and pelvic portions of the digestive tract and its associated organs, the spleen and the pancreas, these vessels unite to enter the liver as the portal vein which breaks up into the capillary system within the liver before collecting again as hepatic veins to enter the vena cava inferior.

Arteries and Veins of the Lower Extremity and Pelvis. (Figs. 48-49.) The common iliac arteries, terminal branches of the aorta, generally divide within the pelvis into external iliac and hypogastric (internal iliac) arteries, but this branching is very variable in the rat. As was seen in one instance there may be no splitting into hypogastric and external iliac (in which case all pelvic branches arise from the common iliac), or the splitting of the hypogastric and the external iliac elements may occur so high that all pelvic arteries spring from the hypogastric artery or trunk.

The pudic-epigastric trunk is the only artery arising from the external iliac above the inguinal ligament. In addition to the pudendal and inferior epigastric elements this branch includes the deep circumflex iliac, and generally the external spermatic in the male.

The hypogastric artery or trunk (internal iliac artery) leaves the common iliac at the level of the sacro-iliac joint. It is not divided into anterior and posterior trunks as in man but its branches may be classified as visceral and parietal. The relationship of these branches as they leave the trunk varies greatly.

Visceral branches	Parietal branches
Superior vesical	Medial femoral circumflex
Inferior vesical	Inferior epigastric
Deferential—in the male	External spermatic
Uterine—in the female	Inferior external pudendal
Vaginal—in the female	Lateral femoral circumflex
Middle hemorrhoidal	Inferior gluteal
Internal pudendal	Superior gluteal

Of particular note is the fact that the lateral and medial femoral circumflex vessels which, in man, arise from the femoral artery below the inguinal ligament, are, in the rat, branches of the hypogastric and arise above the ligament. This is undoubtedly associated with the quadrupedal position in which the hip joint is flexed bringing the thigh close to the trunk and thus making it possible for vessels arising within the pelvis to pass directly into the thigh.

The external iliac vein, the continuation of the femoral vein above the inguinal ligament, receives the pudic-epigastric vein with tributaries following the branches of the corresponding artery, then unites with the most lateral of the pelvic veins, the medial femoral circumflex to form the common iliac vein.

A hypogastric vein as such was seen in only one specimen. Veins corresponding to the branches of the hypogastric artery do not as a rule combine into a single venous trunk but enter the common iliac vein independently or combined with one other vessel.

The common iliac vein (Fig. 48) receives the hypogastric elements, namely, superior vesical, obturator, medial femoral circumflex, lateral femoral circumflex, superior gluteal and internal pudendal. The inferior gluteal joins the latter.

The external iliac artery begins where it separates from the hypogastric trunk and extends to the point where it crosses the inguinal ligament to become the femoral artery of the leg.

A pudic-epigastric trunk is often present in the rat as a branch of the external iliac. In man the pudic elements of this trunk arise below the inguinal ligament.

The femoral artery may be exposed just beneath the skin to a point about half way down the medial surface of the thigh. Beyond this it takes a deeper course passing between adductor brevis and caudo-femoralis muscles to the popliteal fossa where it becomes the popliteal artery for a short distance before dividing at the anterior margin of the popliteus muscle into anterior and posterior tibial arteries.

Two or three muscular branches of the popliteal artery are sufficiently large and conspicuous in the rat to warrant attention at this point, namely the superior muscular and external and internal sural arteries. The first of these, largest branch of the popliteal, emerges from between the hamstring muscles to which it gives branches. It also sends a superficial sural branch down the lateral surface of the lower leg before turning upward along the back of the thigh as the femoropopliteal artery.

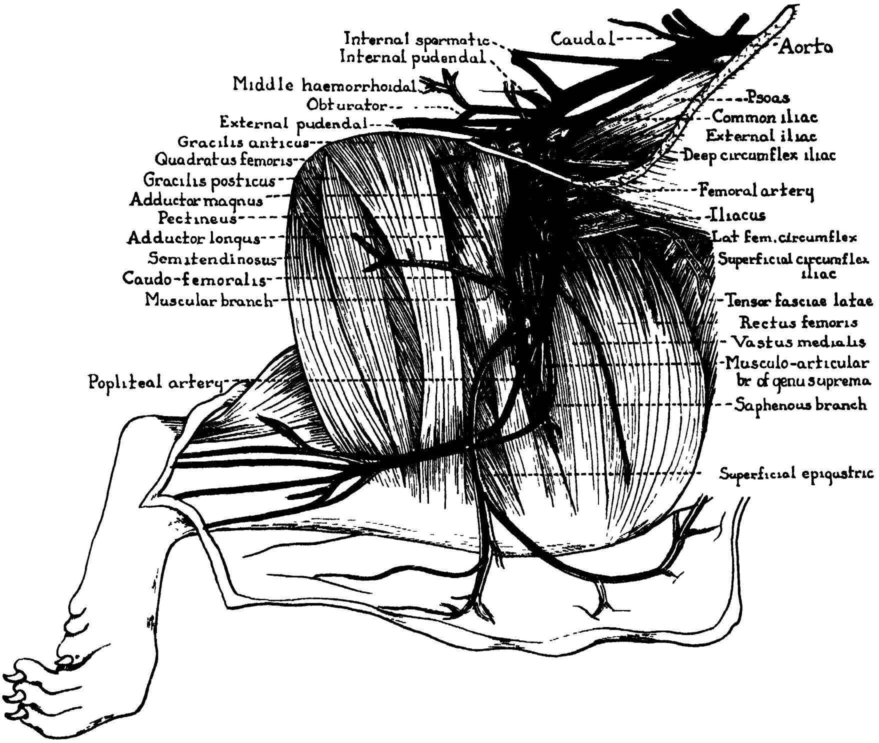

Fig. 49. Femoral Artery and Vein.

The external and internal sural arteries frequently leave the popliteal as a common trunk but vary somewhat in their origin. The external sural supplies plantaris, soleus, and the lateral head of gastrocnemius, while the internal sural supplies the medial head.

The anterior tibial artery takes a deep course along the lateral surface of the interosseous membrane running finally beneath the annular ligament of the tarsus to end as the dorsal artery of the foot. The anterior tibial is a relatively larger artery in the rat than in man since it takes over several branches which in man arise from the posterior tibial, the peroneal artery being the most important of these.

The posterior tibial much reduced in size, runs deep to the popliteus muscle, passes between flexor digitorum longus and flexor hallucis longus supplying each of these muscles as well as tibialis posticus.

Venous tributaries from the plantar surface of the foot and the medial surface of the lower leg unite to form the great (large) saphenous vein at the posterior border of the medial hamstring muscles. Crossing these muscles superficially, the vein enters the femoral vein about half way up the thigh.

The anterior tibial vein from the dorsum of the foot runs with the corresponding artery along the lateral surface of the shaft of the tibia, receives the peroneal vein and joins the posterior tibial to form the popliteal vein.

The peroneal vein also from the dorsum of the foot, follows the peroneal artery and enters the anterior tibial vein which in turn joins the posterior tibial to form the popliteal vein.

The popliteal vein, formed by the anterior and posterior tibial veins, runs with the popliteal artery through the popliteal fossa. It becomes the femoral vein of the thigh where it receives the small saphenous vein.

The femoral vein becomes superficial in the upper half of the thigh with the femoral artery and passes under the inguinal ligament to become the external iliac vein. In the thigh it receives the great saphenous vein and tributaries corresponding to the branches of the femoral artery.

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4

Experimental Methods and Rat Embryos

By

J. S. NICHOLAS

IN THE past many sporadic papers show that the interest in mammalian development and its separate and distinct problems is a real and vital one. The difficulties which have been encountered in attempting to secure an adequate and complete picture of what is occurring have proved bewildering and complex. In many forms nothing but a partial attack was contemplated and therefore the small fragments only could be considered, not the total problem of mammalian development.

Within a single year (1925) papers appeared from three different laboratories, all dealing with phases of experimental observation of fetus. Swensen's[1] dealt with the study of the initiation of fetal movements. Bors[2] and Nicholas[3] with the effect of intrauterine operations upon the subsequent life history of the fetus. These papers accentuated the idea that many investigators have had—that at least a part of the method so successfully used with amphibian and fish embryos might be applied to the more intricate and puzzling field of mammalian development.

It is interesting that all the investigators followed much the same method and used rodent material. The reasons for this are fairly obvious since if a fair frequency of positive result is to be obtained a laboratory animal must have a fair degree of consistency of reaction. While this has in many cases been assumed for any animal in the laboratory, the assumption has led to many errors, for in any research involving complex technic, the animal is the critical link. Without a knowledge of its background all technical procedures are a matter of chance and not of precision.

The work of Huber[4] on the development of the rat gave the experimental embryologist an accurate and complete description of the early stages up to the acquisition of embryonic form. The larger problem is to tell how each event happens and the underlying causes which accelerate or retard these events. The adult animal has been investigated so thoroughly and conscientiously by the Wistar group that its reactions and standardization are well known. The immediate question which faced the experimenter at the beginning was the fairly obvious one: how will the normal course of pregnancy be affected by operations performed upon the mother at various stages during the cycle? It was necessary that this question be answered before any embryonic manipulations were carried out. The preliminary survey showed that with ordinary care, the rat would survive the necessary laparotomy without perceptibly altering the course of events necessary for the production of young. As the problem yielded a solution to this first point of attack the next step was already in sight: is it possible to go through the wall of the uterus and the embryonic membranes to operate upon the fetus and still secure normal young? This question was also to receive a positive answer but the operative procedure was limited to the last third of pregnancy for reasons which will be presented below. Suffice it to state here that during the second third of pregnancy, the relation of the decidua to the embryo is so intimate that only rarely can an adequate environment be reconstituted for the embryo after the necessary manipulations have been completed. It is for this reason that the development occurring during the second third of pregnancy has had to be investigated by methods of tissue culture. It has also led to a complete restudy of the decidual reaction and the normal relationships of form development during those critical stages about which we have so much to learn.

The first principle which, peculiarly enough, must be laid down for anyone who wishes to work upon mammalian embryos from an experimental viewpoint is that every possible point which might influence the physiological reaction of the mother and through her the development of the young must be carefully considered. In institutes having large colonies most of these factors have been so adequately controlled that the ordinary routine takes care of all important details. For a beginner, however, where the colony is small and the routine of animal care does not seem so vitally important, there are frequent upsets which can easily ruin many experiments. There is no one individual point which an investigator can mark out as absolutely necessary for there is no way by which specific rules can be laid down. The rules as determined at the Wistar Institute after years of experience seem to cover all general considerations. Specific rules are generally misleading and sometimes really dangerous.

In our colony at the Osborn Laboratory the following are regarded as cardinal necessities: (1) regularity in care, (2) a temperature which has not too wide a fluctuation, (3) cages of metal which can readily be cleaned, (4) feeders filled with dog or fox chow, (5) plenty of air space and cleanliness. This is a basic minimum and to it is added a supplement to the diet by feeding of fresh greens two or three times a week. It will be found that this simple treatment given regularly will keep any colony in a good standard condition—its simplicity is its index to a normal standard. If a scrap diet is used, as is frequently done, more care is necessary and this diet which initially seems cheap may, if infections are introduced, prove very costly. The nearer the dietary conditions can be brought to a simple regular mean, the more nearly standard are the resulting animals from an experimental standpoint. Detailed descriptions of care and breeding will be found in an earlier chapter.

The anatomy of the female is adequately described in Chapter 3 and in Long and Evans.[5] The landmarks for operative approach to the female genital system are clearly designated in Chapter III (Figs. 36-37). The correlation between external morphology and ovarian reaction is set forth in Long and Evans.[5] The reader is referred to these two works, a knowledge of which is a prerequisite for experimental work. The first is necessary for the spotting of landmarks for operation, the second gives in detail most of the difficulties which must be overcome if an investigation upon the embryo is to succeed. The primary requisite to the approach to the embryo is a study of the estrus cycle of the female. Only by having an adequate knowledge of the external signs and their relation to internal function can an adequate approximation to embryo age be secured. This knowledge is requisite for the timing of the embryo and even with all safeguards taken this proves sometimes only too variable.

The estrus cycle as described by Long and Evans extends over four days and is divided into five periods. In most cases our experience has shown that the cycle extends into and frequently through the fifth day. The separation of these five stages depends on the examination of the vaginal content and its correlation with ovarian and uterine changes. These changes are shown in summarized form in Table III.

TABLE III

Rat Estrus Cycle (Long and Evans '22)[6]

Stage	Superficial genitalia	Vaginal smear	Uterus	Ovary	Length	Remarks
I.	Lips slightly swollen Vagina dry	Epithelial cells only	Increased distension fluid	Ovarian growth. Follicle enlargement	Ca. 12 hrs	Heat may occur
II.	Lips swollen Vagina dry	Cornified cells only	Maximum distension early. Regression	Large follicles. Maturation of egg	Ca 12 hrs.	Heat Copulated
III.	Lips still swollen, regressing. Cheesy mass in vagina	Cornified cells only	Epithelium degenerating	Ovulation	Ca. 15–18 hrs.	Not in heat
IV.	No swelling. Mucosa moist	Cornified cells and leukocytes	Begins regeneration	Eggs in oviduct	Ca. 6 hrs.	
V.	No swelling. Mucosa moist	Leukocytes and epithelial cells	Epithelium regenerated	Corpora lutea formed	Ca 57–60 hrs.	Estrus

Copulation may occur in late Stage I, in Stage II and occasionally in early III. Ovulation may occur in late Stage II and later. Copulation time usually precedes the ovulation, the limits so far found are 6–30 hours. Copulation plug forms 3–8 hours after copulation and is not by itself an adequate criterion of timing.

The material used for designating stages of estrus is subject to wide individual variation and these variations may be increased in number by the investigator unless care in handling and interpretation are used. As Long and Evans have stressed, the insertion of a glass rod into the vagina may upset the next occurring cycle and may even extend further and produce a pseudopregnancy with a duration of twelve to sixteen days before the re-establishment of the cyclic condition. Rough handling during examination or the taking of smears may produce the same condition. The criteria for each of the stages must of necessity be arbitrary. With this arbitrary staging the individual animal sometimes fails to agree. In addition there are physiologic overlaps in the stages which obscure the picture and investigators have sometimes felt that the smear method is more confusing than accurate. The addition of additional stages by some workers does not clarify the picture but adds confusion. Normal stages can always be fractionated for specific ends but the simple staging as given above is probably the best obtainable. The individual points are subject to wide and peculiar variations but with careful study of the animal through several cycles the specific variations become clear. One point or another may vary but the complete picture is fairly uniform and subject to a rational interpretation. No one can expect the animal to follow all the rules; we are fortunate that the story is as readable as it is.

The smear must not be taken alone as diagnostic of the ovarian condition. Recently another method has been applied to the study of ovulation, the study of the changes in electropotential occurring during this period. Burr's work[6] has shown that this can be used in rabbits and the human cycle. It is also indicated that the same condition obtains in the rat. The author has repeated this work on the rat and has secured definite information that there is a change in electropotential which can be correlated with ovulation and also with the various stages as indicated by the vaginal smear. Considerable information has also been acquired as to this reaction during pregnancy. These will form a future report.

Any method by which a small portion of the vaginal content can be obtained without injury to the animal may be used. The easiest way which we have employed is to use a small cotton pledget moistened with physiologic saline. The animal is held loosely in the left hand while the vaginal margins are separated and the pledget introduced. The cotton may be held in forceps or a small tampon may be made with a toothpick. The importance of moistening the cotton with saline is that it then collects the vaginal content with the least irritation. Some investigators when taking smears try to collect all the available vaginal secretion. This not only is unnecessary but dangerous if succeeding smears are to show anything about normal cycles. The material at the lips of the vagina is just as diagnostic as that near the uterine os. It is unnecessary to curette the complete vaginal content as many do by inserting dry cotton and then twisting it to get what they regard as the necessary amount of material. Large quantities are not necessary for accurate diagnosis. Use care, avoid roughness and smear diagnosis becomes more accurate and less confusing. Washing the vagina with physiologic saline is a good method. It requires a steady hand, however, or uterine stimulation will result, particularly if the animal struggles during examination. When carefully used it is as gentle as any other method of examination.

Any stain can be used which will give the cell picture. I prefer Wright's stain because of its simplicity and its clear diagnostic differentiation. Hemotoxylin and eosin are just as satisfactory but require more care in handling. Where a large number of specimens are to be examined, Wright's stain is a time-saver.

All the foregoing is a necessary background for timing the embryos. Throughout the literature timing has had various points of reference. The age of the embryo cannot be absolutely determined by the time of copulation, ovulation, or fertilization. Only the first can be observed and its effectiveness depends on concomitance in the female cycle. Ovulation is also manifested electrically by a change in potential so that we may be able to say that this incident is also observable. We still, however, lack direct information on the fertilization complex which in itself shows an astounding variability in individual cases. It is remarkable that the various investigators have come so close to a normal timing. This in turn has depended more on the embryomaternal relation than might be supposed, for minor irregularities are smoothed out during development by a series of regulatory processes. This results in the retardation of the more advanced development and a regulation of the more slowly developing.

The potency of the immature egg to develop after fertilization has been shown by Pincus.[7] In the rat at the time of ovulation the eggs are in various stages of maturation. This is clearly shown by the variability in cleavage rates. It is frequently the case that uncleaved, two, three, and four-celled eggs are found in the tubes of a female forty-eight hours after copulation. Sometimes the uncleaved eggs will make up the larger proportion of the product obtained. Since Long and Evans[5] have shown that approximately one third of the ova at ovulation do not develop to term it is probable that many of the uncleaved, probably immature eggs do not complete development. The possibility, however, always exists that some eggs may not have started development forty-eight hours after ovulation. This is an extreme case, but it is not unusual. In the light of these facts alone, it is hard to realize how some investigators have recorded the timing of pregnancies to the fraction of an hour. The precociously developing blastocysts are held in the tube until the opening of the tubo-uterine junction. This may occur somewhat earlier than described for it has been found patent and expanded eighty-four hours after copulation although it usually remains closed until ninety-six hours. During this period some of the eggs show little or no development and some show definite degenerative changes resulting in fragmentation. The viable although retarded eggs tend to be slightly accelerated during this period and at the end of ninety-six hours are definitely advanced over their original condition although not yet equal in development to the most advanced. The first eliminating process is thus the fragmentation of eggs which were immature or otherwise unfertilizable at ovulation.

The second elimination occurs in the uterus for not all of the fertile eggs become implanted. As is well known from the work in production of deciduomata, the rat uterus responds to a number of stimuli in addition to those initiated by the egg. Electrical and mechanical stimulation as well as any foreign body introduced into the uterus at this period will bring about pseudopregnancy with the cessation of the estrus cycles for twelve to fifteen days. A two-celled egg can be transplanted to the uterus and develop normally although it is early by three days for this environment. The uterus is an admirably protective agency at the time the eggs enter the uterus and lodge in the antimesometrial mucosa. Even a single blastomere will survive and become implanted normally in the uterus.[8] If, however, the egg fails to undergo requisite concomitant development the decidual overgrowth results in early resorption. Blastocysts may be retained for several days in the uterus without implanting and still survive as shown in Pincus's experiments[7] with theelin retardations of implantation.

These regulatory processes then tend to produce embryos of the same stage at about the ninth day after copulation although small individual variations can still be detected even in the most regular development. One of these occurred in one series of experiments in which the embryo (10 days development) showed nine somites on one side, three on the other although all other organs were normally and regularly formed. This is just one instance of variability which gives a quite different picture from that which we usually have of the embryo. The third period of regulation is the time when the placenta is just beginning to function for the maternal blood stream supplies a proportionately higher amount of blood to smaller embryos. The differentiation rate seems to be maintained even when the growth rate is retarded. The fourth period of regulation is that just before parturition when the larger fetuses are distinctly slowed in growth and those slightly retarded approach the growth total of the larger. These four regulative periods may well be referred to as the equalization factors in rat development. Their existence simply makes us more aware of the necessary inadequacy of our timing methods.

The cardinal steps which are correlated with the normal development of the embryo may be roughly although incompletely sketched as follows:

Copulation—Estrus stage II
Copulation plug, 8-20 hours = ovulation
If eggs are mature and sperm viable = fertilization
1st cleavage, 2 cells, 24 hours after ovulation
2nd cleavage, 4 cells, 48 hours after ovulation
3rd cleavage, 8 cells, 72 hours after ovulation
4th cleavage, 96 hours after ovulation
Early blastocyst, relaxation of tubo-uterine junction; entrance to uterus. 80-108 hours after copulation.
Implantation, beginning uterine decidual reaction, 5 to 6 days. Uterus may respond to implants 2 days after copulation.
Completion of decidua with orientation of blastocyst. Formation of ectoplacental cone. Blood vessel enlargement with formation of periembryonic capillaries: 6-7 days after copulation.
Development of differentiated germ layers; 8-9 days after copulation.
Development of yolk sac epithelium.
Differentiation of contorted embryo.
Histogenesis, dominantly nervous system and mesoderm.
Embryonic heart beat and intra-embryonic circulation.

Nervous system partly closed, embryo rotating in amniotic sac, extension of allantoic stalk to embryonic placenta.
Growth and differentiation with heart chambers, completed closure of nervous system and formation of first gut structures. Functional placenta.
Embryonic movements, placental extension.
Growth, mainly gut, embryonic hernial loop.
Form completed, all subsequent differentiation confined to parts, growth mainly in size.

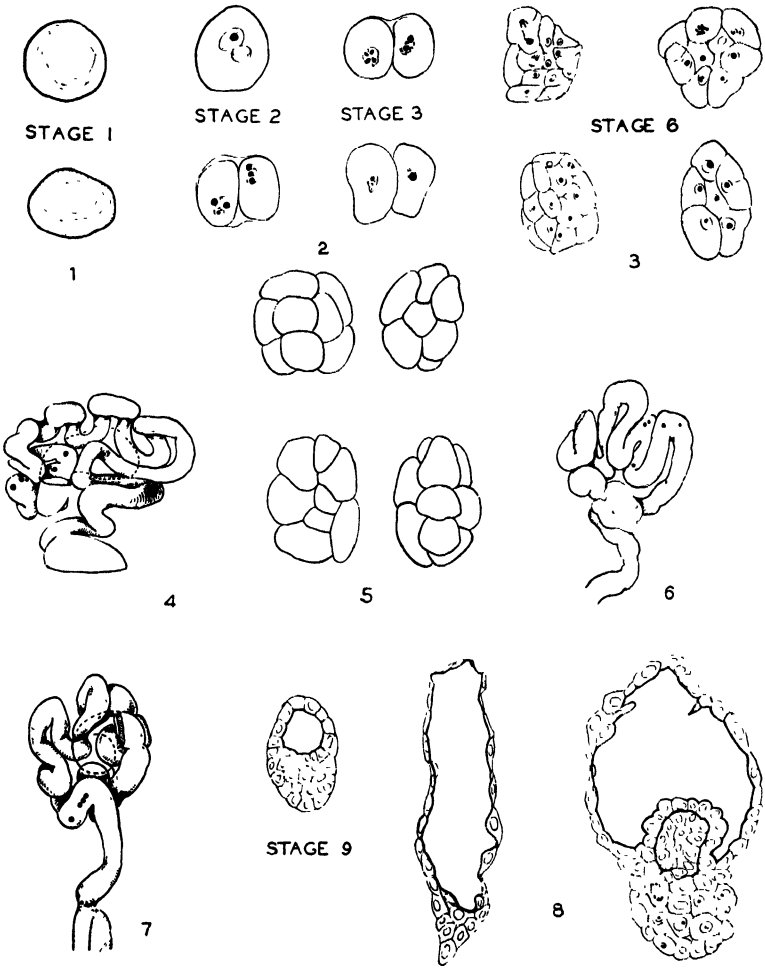

Fig. 50. Early stages of the rat embryo.

These events mark out the main developmental highlights which, however, have been differently classified by the various embryologists who have worked upon this problem. In order to coordinate the previous work and make it usable for the experimentation it has been necessary to standardize the embryonic development in a normal series consisting of forty arbitrary stages.

Stages 1-10 are segmentation stages up to the formation of the blastocyst (Fig. 50). The time interval covered in these stages is approximately five days after fertilization.

Stage
11. Beginning egg cylinder (8th day)
12. Cavity formation
13. Primitive streak; amnion (9th day)
14. Neural plate
15. Head folds; allantois
16. First somite pair (10th day)
17. 2 prs. somites
18. 3 " "
19. 4 " "
20. 5 " "
21. 6 " "
22. 7 " "
23. 8 " "
24. 10 " " (11th day)
25. 12 " "
26. 14 " "
27. 16 " "
28. 18 " "
29. 20 " " Fore limb bud
30. 25 " "
31. 30 " " Tail growth
32. 35 " "
33. 40 " "
34. 50 " "
35. 60 " " (360 hours)—
36. 65 " " Motor reactions
37. 16 day
38. 17-18 days
39. 19-21 days
40. Parturition

Accurate designations of time must be avoided; those listed are simple approximations which help to correlate the stage of development with the results of other workers.*

At Stage 11 the egg cylinder is a delicate elongated capsule enclosed in the decidual shell (Fig. 51). The cylinder itself is composed of two tissue layers; a central cavity forms during the next stage (Stage 12) and the subdivision of the embryonic region from the rest of the cylinder is foreshadowed by a pinching of the internal walls of the cylinder.

Sometime during the ninth day of development the amnion as a membrane becomes complete, and mesoderm proliferation begins from a lateral region of the antimesometrial portion of the cylinder, between the two layers. (Fig. 52.) This stage (13) is designated the primitive streak, although the streak itself is not prominent in surface view. In Stage 14 the medullary plate is distinct, a notochordal rudiment is present, and an endothelial sac anterior to the medullary plate marks the appearance of the heart. In Stage 15 the anterior end of the medullary plate is elevated to form the large bilobed head fold; the foregut invagination appears; and an allantoic bud is found at the posterior end of the axis. The capsule as a whole greatly increases in size at this time.

At Stage 15 the embryonic axis is practically a bilaterally symmetrical sector of the capsule; shortly thereafter the primary torsion begins in the region of the anterior intestinal portal and the first somites—that is to say, at the lowest point of the embryonic capsule, and its point of greatest curvature. During this time the amnion has changed in form from what was practically

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* Too frequently embryologists have been unaware of the biblical standard "that the evening and the morning constitute the *first* day." An embryo on the 9th day of development is really 8 days old.

a plane surface cutting off the embryonic end of the cylinder, to an almost independent sphere or ovoid, contained within the capsule, but touching it and the embryo only at its point of origin at the lower pole, i.e., the intestinal portal. The allantois very soon grows downward to join the cone of the forming placenta. While these changes in general form occur, the embryonic body has been detaching from the capsule, and is undergoing rapid differentiation and growth, at the same time turning over on its back. The medullary plate, almost as soon as the headfolds appear, becomes precociously folded into the primary brain divisions; this process precedes by several stages the closure of the plate in the head region. In the trunk, the medullary tube closes much earlier than in the head; in the tail region it is formed directly, without a plate stage. The original two- or three-layered pseudostratified medullary epithelium becomes much thickened while these primary morphological changes take place; an ependymal layer forms and stratification of the wall begins. The mesoderm during these stages is a dense mass, filling the body; somites are split off the segmental plates as the tail extends posteriorly, and the cells immediately assume the radial arrangement characteristic of myotomes. The heart begins to function very soon after Stage 15, and both yolk-sac and embryonic circulation are rapidly established. The hindgut is formed soon after the foregut; both of these structures early become elongated tubes of characteristic form.

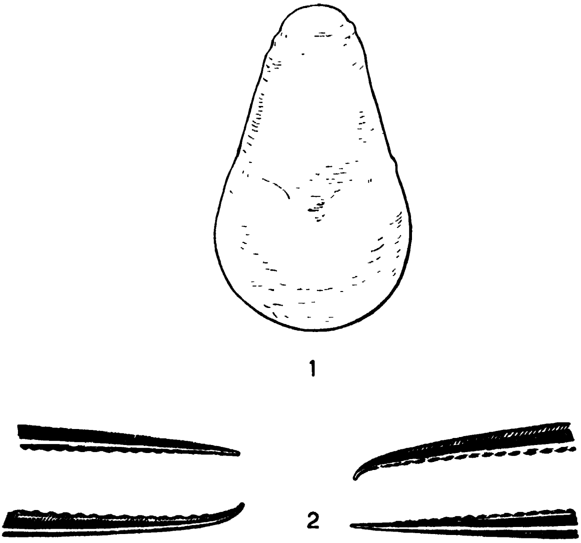


Fig. 51. Stage 11 of the embryo.

With the foregoing account of the normal development in mind the experimenter can roughly divide pregnancy into three periods according to the type of problem which he desires to attack. The first of these takes in the problems of fertilization, segmentation, migration and implantation and includes the first seven days of pregnancy. The second period of seven days includes the formation of embryonic membranes, the differentiation of the germ layers and their organization, the turning of the embryo and the formation of the functional placenta. The third period begins with the development of nervous reactions and is completed at parturition. The latter period is one which appeals most strongly to the students of functional relations, the first period has up to the present received scant attention from those who should be attacking the correlation between the maternal endocrines and development. The second period is the one in which the experimental embryologist finds the greatest likelihood of ascertaining the factors leading to the acquisi-

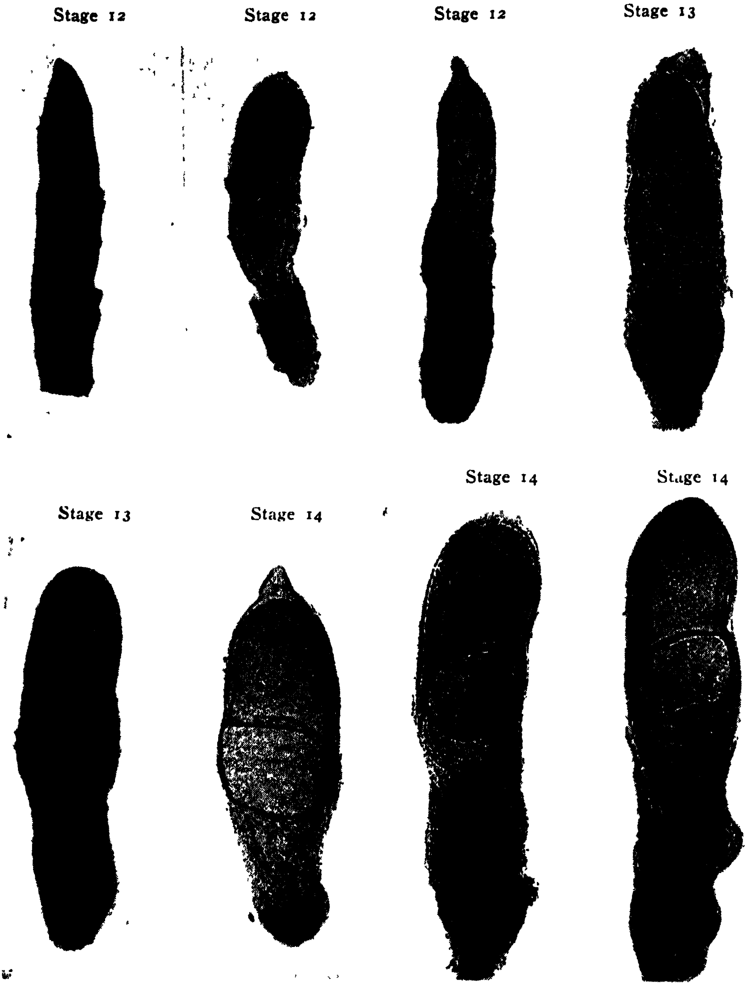


Fig. 52. Stages 12 to 14 of the rat embryo.

tion of form and function. Having ascertained the period in which the investigation is to take place the resulting approach is fairly easy.

A schedule is kept of the estrus cycles of the females and those with normal cycles are mated.[5] Animals can be mated in late Stage II of estrus with a fair degree of accuracy, and the early formation of the copulation plug observed. The pregnant female is anaesthetized with sodium Amytal, depilated ventrally with sodium sulphide, and washed with strong chlorozone solution. The abdomen is opened from a point in the ventral midline just above the symphysis pubis to approximately the umbilicus by a single incision and the abdominal walls retracted by means of a Beard's eye speculum. The animal

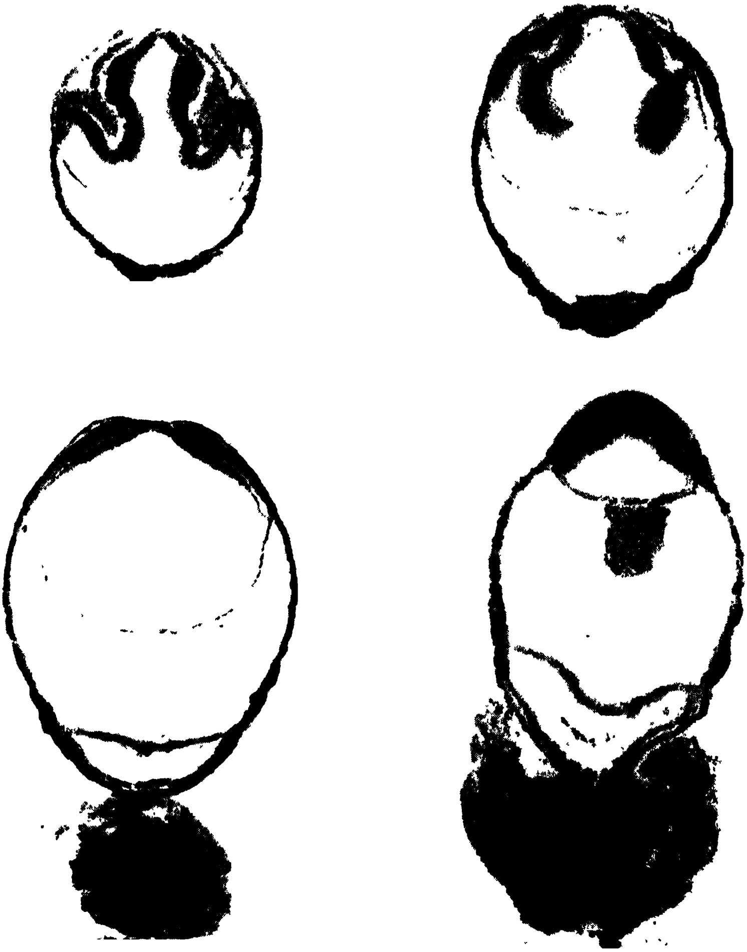
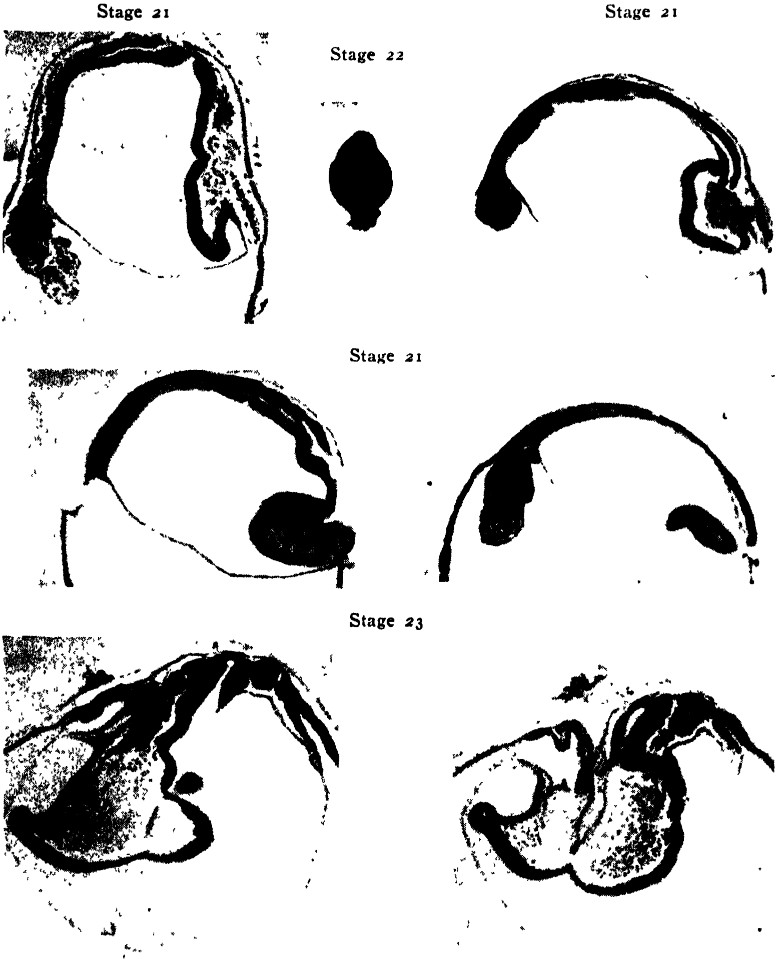


Fig. 53. Stage 15 of the rat embryo.

is covered with a sterile white rubber sheet, through which this operation is completed. The uterine horns are grasped with fine forceps and delivered through the opening, being raised to a position on top of the sterile sheet for examination. Mosquito hemostats are clamped upon the lower portion of the


Stage 21

Stage 21

Stage 22

Stage 21

Stage 23

Fig. 54. Stages 21 to 23 of the rat embryo.

uterus and also upon the ovarian mesentery. Any subsequent bleeding will consist of residual blood. Fine forceps are used to grasp the muscular coats of the uterus through the serosa at the antimesometrial margin of the decidua. This being lifted, a cut with a fine pair of scissors completes a circular opening in the uterine wall through which the embryo in its decidua can be delivered. Delivery of early stages (Stages 10-15) in their complete membranes is simple; care, however, must be used with Stage 25-30 embryos, since the embryonic membranes are thin at this stage, the decidual covering also is thinned at the antimesometrial pole, and the embryonic sacs can easily be ruptured and the embryo extruded.

The decidua is detached from the mesometrial portion of the uterine mucosa; this causes an inconsequential residual bleeding. The decidua is pear-shaped with a slight ridge around its mesial part. The lateral portions of this ridge dip downward and are in contact with the walls of the uterus. At the center of the depression in the ridge there is a very slight aperture.

The deciduomata are placed in sterile warm Ringer's solution, washed to free them from blood and placed in fresh warm Ringer's solution for the dissection of the embryo. This is performed under a binocular microscope with the aid of special cutting forceps. The decidua is turned until the lateral portion of the decidual ridge, as determined by the decidual aperture, is toward the observer; the cutting forceps are applied near the base of the decidua. When gauged correctly, the decidua can be split accurately into its two halves. The embryo in its covers can then easily be loosened from its remaining contact with the decidua, beginning in the region of the ectoplacental cone in younger stages, in the region of the forming or formed placenta in the older ones. The embryonic membranes and the yolk sac epithelium are kept completely intact. The embryo is staged accurately at this time.

The continuity of the external membranes is a fair index of the state of the embryo. If they are not continuous, the outer layer yolk sac epithelium peels away from the embryo which protrudes through the break. By cutting the top of the cone (a combination of Reichert's membrane and the transitory or extra-embryonic ectoderm) the embryo is exposed. The membrane retracts toward the base and remains attached to the ectoplacental cone leaving the embryo free for operation.

The embryo is situated at the upper pole of the cylinder, which may be considered as consisting of three parts during Stages 12-15: the mesometrial third, made up of the ectoplacental cone; the middle third including the false amniotic cavity; and the upper or antimesometrial third which contains the embryo and is sharply marked off from the other parts by the amnion. The midpoint of the antimesometrial pole marks the connection between the embryo and the yolk sac epithelium. As the embryo develops, it rotates through 180°, assuming what is considered to be its normal orientation through the action of a series of complex factors, i.e., growth, distention of the left vitelline vessels, and establishment of placental connection. Later in development the orientation again changes and during the eleventh, twelfth, and thirteenth days the rat embryo lies upon its right side, i.e., the right side of the embryo is toward the placenta, the left toward the antimesometrial portion of the membranes.

The procedure will naturally vary according to the problem in view. For cauterization experiments or spinal cord operations the dorsal approach may be used. For obtaining eggs or for work on the Fallopian tubes the ventral approach is better. The ventral approach should be avoided if it is desired to have the litter born and suckled since this frequently upsets lactation. Rigorous care is necessary to keep the instruments clean, sterile solutions are used at every point in the operation, dishes and sponges are sterile. With all of these precautions, however, the operations are far from aseptic but the resulting reactions of the mother seem to show that there is not sufficient infection to cause serious trouble. Adhesions of the mesometrium to omentum and mesenteries show at subsequent operations that local infections were an accompaniment of the operation preceding but these are not severe enough to cause reactions on the part of the mother nor do they inhibit succeeding pregnancies.

There is a possibility, fortunately rarely occurring, that the embryo and its coverings may be infected by the flora of the female genital tract. This is to a large extent taken care of by washing the deciduomata in sterile physiological saline several times before opening the capsule. After removal of the capsule the embryos are washed in several changes of saline before further treatment. After such treatment one may be fairly confident that bacteria have been eliminated unless there is a massive infection which has been brought over from the uterus. When such a condition obtains all cultures or transplants are unsuccessful. This seldom happens, so that the infective material carried over at the time of experimental procedure can be regarded as a negligible factor in any series of experiments performed with reasonable care. It is surprising how rarely an aseptic condition can be obtained if cleanliness is rigorously controlled.

The handling of the materials varies according to the stage of the material used. The treatment for eggs is different from that for blastocysts. The easiest way to secure rat eggs is that given by Lewis[9] and so successfully used in their studies on the mouse egg. After a study of the normal cycles the formation of the copulation plug is observed and the events later correlated with this time. More recently a more positive timing of ovulation has been obtained by the determination of the electropotential which places in most cases the time of ovulation as 8-16 hours after the formation of the plug. The Fallopian tubes are then excised and cut into small pieces in sterile physiologic saline contained in an embryological watch glass. The container and contents are kept at 35-37°C on a warm stage and then later examined for eggs under the binocular dissecting microscope. A fair yield can usually be obtained, eight to ten eggs of the desired stage. As was schematically given above, cleavages occur fairly regularly each 20-24 hours. Most work of an experimental nature in our laboratory has employed the two-celled stage since here one has the greatest evidence of regularity. The egg is normal and has demonstrated its viability. Atypical and irregular eggs should be discarded; they usually can clearly be recognized at this stage.

The egg can be transferred to other solutions either with hand or mouth pipette, it is stable for periods of several hours and has survived long periods in physiologic saline. This, however, should be made of as short duration as possible as it introduces factors which complicate the experiment. Two sets of egg transfers have been made indirectly. In one of these the operative procedure involves the manipulation of the uterine horn either by cutting and ligation or by substitutive transplantation. In the former case the uterine horn is ligated 24 hours after copulation and the eggs dropped into the abdominal cavity where development ensues on the mesometrium. This is one method of producing abdominal pregnancy for study.[10] In the second case a segment of gut is substituted for a segment of uterus and the eggs implanted in the intestine after erosion of the musoca. In both cases development proceeds normally with regard to the form of the embryo. The second situation, however, deletes factors which limit development and increases the rate of growth to a large degree.

In the direct method the egg is transferred by means of a pipette to the region to be tested for embryo maintenance. This method has been used in studying the developmental potency of the egg in various locations. The transfer system so far has been most successful when the egg has been located either in the uterus of the same, or of a similarly timed mother, or under the kidney capsule. In the uterus the egg develops into an embryo with normal form and size, under the kidney capsule all parts undergo precocious histogenesis but the tissues and organs never form an embryo. This has led to numerous experiments which, however, have not been sufficiently critical to explain all the differences although they tend in general to show that the mechanical and nutritional factors found in the uterus or in surrounding structures must be inhibitive to some degree and that this inhibition is partly responsible for the correlation of structure and the production of form.

So far attempts to grow rat eggs in tissue culture preparations have been unsuccessful; it has been impossible to grow them as grafts in the eye, on the mesentery, on the chick chorioallantois, or in the mammary gland. In the light of Pincus's experiments with rabbit eggs it may be possible with additional information to cultivate rat and mouse eggs. I am convinced that the difficulty is technical and not due to low viability or resistance to handling peculiar to rat material.

Blastocysts are secured by washing the uterus at or shortly after the opening of the tubo-uterine valve. It is difficult to catch this material in the relatively short time between its entrance into the uterus and its implantation. This process is a matter normally of very few hours but the period can be prolonged by injections of theelin.[7] Washing of the uterus is accomplished by inserting a small glass cannula into the uterus near the tubo-uterine valve and gently injecting and withdrawing fluid. In this way blastocysts can be secured and the animal will carry through any which may have become implanted. A second method is the complete removal of the uterus which is opened by an incision along the mesometrial side. This is performed in physiologic saline and with the uterus fastened to the floor of a wax dish. With a mouth pipette a stream of physiologic saline is directed against the antimesometrial mucosa loosening the blastocysts. I have never secured more than two or three blastocysts by this method.

After implantation the decidua forms rapidly. In the early stages it is exceedingly gelatinous and because of this the embryo is separated from the decidua with great difficulty. The egg cylinder at this stage resembles a miniature baseball bat with the ectoplacental cone at the handle and the embryonic pole at the distal (antimesometrial) end. The decidua is much more easily separated from the embryo in later stages as given above.

Succeeding stages offer less difficulty in the removal of the embryo and its membranes if suitable precautions are taken in handling the tissues. The decidua thins considerably during the development of the 9, 10, and 11 day stages and the yolk sac epithelium which is earlier found only at the upper margin of the ectoplacental cone now thickens and extends completely over the embryo. The maternal blood vessels invade the yolk sac epithelium and set up an active circulation. This is at its maximum at 11-12 days and bridges the period during which the true placental mechanism is developing and coming into function. The yolk sac epithelium is really a precocious placental mechanism in the rat.

Operations in utero at this stage are not feasible for the fluid pressure within the membrane results in the total expulsion of the embryo. It is for this reason that experiments at this stage must be combined with tissue culture methods. Törö[11] has given an example of how this combination can be applied. It does not always give as good an experimental environment as does the uterus but it has the advantage of permitting manipulation which cannot successfully be performed in utero.

Operations in utero may be performed successfully subsequent to the 15-day stage. This is just about the time of the first nervous reaction. Studies on the regeneration of the nervous system, injections of various hormones, and extirpation of the adrenal are but a few of the studies to be completed along this line. Many other problems are open to attack by this relatively simple method. The embryo is fairly large and is plainly visible through the dilated uterine wall. With a small incision made in the uterus after its exposure the fetus can be brought into position for operation. The fetal anatomical landmarks are quite evident with a little manipulation of the light to give a transillumination. The only thing which limits operation is the placenta which must not be disturbed.

The later the stage of experiment the greater the percentage of viability. This is decidedly different from embryonic transplantations where the largest percentage of viability is obtained with early embryonic material. Nine and 10-day stages afford the best results for tissue culture experiments.

Sufficient has been said to show that the approach to the embryo is a feasible one which can give us information about many of the debatable points in embryonic history. It is to be hoped that this growing field will prove attractive to many investigators who will proceed with the work which at present is in its early pioneer stages.

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5

Dietary Requirements of the Rat

By

RICHARD H. McCOY

THE purpose of this chapter is to present a review of the literature concerning the quantitative dietary requirements of the rat. In addition, typical deficiency symptoms are described and in many cases the normal physiologic functions are indicated. A considerable amount of detail is included concerning the methods employed and the results obtained by the investigators, in order that the reader may better judge the importance of each contribution. No attempt has been made to compile a complete bibliography for any diet constituent, but exact references are given for all the material included in the discussion. A final summary of the data is included at the end of the chapter.

CALCIUM AND PHOSPHORUS

Calcium and phosphorus are discussed together because the adequacy of the intake of either element, in supplying the animal's requirements, depends so largely upon the intake of the other.

Schneider and Steenbock[1] have described the preparation of a synthetic diet very low in phosphorus and free of vitamin D but adequate in all other nutritional requirements of the rat (P = 0.04%, Ca = 0.57%). When young rats are fed this diet they develop rickets in 12 to 14 days and severe skeletal deformities within 6 weeks. Growth stops after 4 or 5 weeks, followed by a rapid decline in weight and death usually by the end of the 6th week. However, when young growing animals are transferred to the low phosphorus diet supplemented with adequate vitamin D, an immediate loss in weight occurs which is not regained for more than 3 weeks. From calcium and phosphorus balances and tissue analyses the authors conclude that vitamin D induces the utilization of phosphorus by bone, thereby depriving the soft tissues of their supply of phosphorus, which in turn inhibits growth. These animals survive for as long as 20 weeks, in contrast with those not receiving vitamin D, and consistently develop urinary calculi.[2]

Day and McCollum[3] have prepared a diet which contains only 0.017% phosphorus but adequate amounts of all other nutrients (Ca = 0.4%). Young rats restricted to this ration grow slowly for 5 to 6 weeks, and then decline in weight and die 2 or 3 weeks later. Extreme rarefaction of the skeleton quickly occurs, accompanied by progressive disability in walking, standing, and breathing. Nitrogen retention is positive for 5 weeks and then becomes negative simultaneously with the onset of loss of weight. The mobilization of calcium and phosphorus from the bones leads to a tremendous loss of calcium (principally through the urine) and a considerable loss of phosphorus (largely in the feces). The greater loss of calcium than of phosphorus suggests that part of the phosphorus mobilized from the bones is taken up by the soft tissues to permit their growth. This severe phosphorus deficiency does not seem to have any significant effect upon the metabolism of sodium, potassium, or magnesium.

Despite the participation of calcium in many important physiological functions, dietary deficiencies of calcium have rarely been observed to produce specific deficiency symptoms. Kleiber, Boelter, and Greenberg[4] have shown that rats receiving a diet containing only 0.010% calcium (P = 0.546%) weigh, after 2 months, about 30% less than their litter mate controls fed ad libitum on the same diet, but with added calcium. The retarded growth is attributed mainly to a decrease in appetite on the calcium-deficient diet. The body weight of the calcium-supplied rats can be kept equal to that of their calcium-deficient pair mates by restricting their food intake to a level slightly below that of the deficient rats (calcium-deficient group ate 456 Gm., calcium-supplied group ate 372 Gm. to permit equal growth). The carcasses of the deficient rats contained only one-half as much ash, and only one-third as much calcium as those of the calcium-supplied controls. On the basis of body-weight gains from the food consumed, the calcium-deficient diet may be said to decrease the efficiency of utilization of food materials.

Sherman and Booher[5] find that with diets of constant phosphorus content (0.42%) increasing the percentage of calcium progressively from 0.16 to 0.50% permits more rapid calcification of the growing body and earlier maturity, and extends the period between the attainment of maturity and the appearance of senescence.

The ratio of calcium to phosphorus in the diet exerts a marked effect upon growth, reproduction, ash content of the bones, and the calcium and inorganic phosphorus content of the blood serum. Shohl and Wolbach[6] conclude that, in the absence of vitamin D, rickets may be produced with not only high-calcium, low-phosphorus and low-calcium, high-phosphorus diets, but also with low-calcium, low-phosphorus rations. This last group occurs in a zone in which the ratios of Ca:P have been called normal, thus demonstrating that rickets may be produced with any ratio of Ca:P in the diet. Bethke, Kick, and Wilder[7] have demonstrated that increasing the Ca:P ratio from 1:1 to 5:1 causes a progressive decrease in growth, bone ash, and the percentage of inorganic phosphorus in the blood serum. Decreasing the Ca:P ratio from 1:1 to 0.25:1 decreases the growth and the percentage of calcium in the blood serum, but has only a slight effect upon the bone ash. According to their data, the ratio of Ca:P in the ration is more important in determining the growth and calcification than the actual concentration of these elements in the food. They conclude that the most favorable Ca:P ratio for growth and bone formation lies between 2:1 and 1:1 (P = 0.56 to 0.69%). Cox and Imboden[8] find that successful reproduction and lactation in the rat are dependent upon both the actual level and the Ca:P ratio of the diet. The best results are obtained on a diet containing 0.49% each of calcium and phosphorus, corresponding to a daily intake of about 42 mg. of each element. Reproductive performance is poor when the diet contains excessive minerals (2.45% of either Ca or P) irrespective of the Ca:P ratio.

Hubbell, Mendel, and Wakeman[9] have devised a new salt mixture for use in experimental diets. During a selected period of very rapid growth (from 60 to 200 Gm. body weight at a rate in excess of 5 Gm. per day) on a diet containing this mixture, adequate calcification occurs with an average daily intake of 50 mg. calcium and 35 mg. phosphorus.

It is generally agreed that inorganic salts of calcium and phosphorus are readily utilized by the animal when included in the normal ration. However, Eddy, Muller, and Heft[10] have shown that the phosphorus of phytin (inositolphosphoric acid), in contrast with inorganic phosphorus, is unable to protect rats receiving a high-calcium, low-phosphorus diet from rickets. Bruce and Callow[11] have confirmed this finding and shown that treatment of the phytin-containing material with 1% hydrochloric acid renders the phosphorus available for the cure of rickets, presumably because of the hydrolysis of the inositolphosphoric acid. Inasmuch as a large part of the phosphorus of grains and seeds is in the form of phytin, these findings are of importance in considering the phosphorus content of the ration. Probably all of the phosphorus of the leaves and stems of plants is available to the organism, for these contain very little, if any, phytin.

The calcium of most vegetables is generally considered to be readily available to the rat. However, several investigators have demonstrated that the calcium of spinach is utilized poorly, if at all, by the rat,[12, 13, 14, 15] apparently because of the presence of oxalates in spinach. Speirs[12] reports that the addition of spinach to a ration diminishes the utilization of the calcium of the milk in the diet. Fairbanks and Mitchell[14] have shown that the calcium of calcium oxalate is not available to the rat.

The requirements for optimum growth, reproduction, and calcification are supplied by a diet containing about 0.5 to 0.6% calcium and the same or a slightly lower concentration of phosphorus. This corresponds to an average daily intake of about 40 to 50 mg. of calcium and 35 to 45 mg. of phosphorus.

POTASSIUM

Miller[16, 17] states that "the minimum potassium requirement (for rats) lies somewhere between 0.55 and 1.44 Gm. per kilo of ration." He concludes that the minimum daily requirement for normal growth is approximately 15.0 mg. for the male and 8.0 mg. for the female. The daily maintenance requirement for a mature animal is not over 2.0 mg. Young rats fail to grow and die within 8 weeks when the daily intake of potassium is 1.0 mg. or less. Sodium will not substitute for potassium in the support of growth on a low potassium diet.

Leulier and Vanhems[18] report that young rats fail to grow and die within 12 to 24 days when fed a diet containing only 0.013% potassium.

Schrader, Prickett, and Salmon[19] report that rats receiving a potassium-deficient diet (potassium content not given) showed "slow loss of weight, short-fur-like hair, cyanosis, abdominal distention and lethargy leading to coma and death (23 days average)." Pathological changes occurred in the intestines, pancreas, kidneys, and heart.

Heppel and Schmidt[20] have shown that a diet containing 0.14% potassium will maintain female rats in positive potassium balance during pregnancy, but the balance becomes negative and the animals lose weight during lactation. When the content of potassium in the diet is increased to 0.503%, the potassium balance of the mother is still positive at the end of the lactation period. Young rats maintained on diets containing 0.015% potassium show very little growth and eventually die. At autopsy a high incidence of hemorrhagic lungs is noted and the small and large intestines are quite flaccid and dilated. On a diet containing 0.015% potassium and 0.28% rubidium "considerable growth of young rats is obtained," indicating that "for a certain time in the growth period of the rat there is some physiological replacement of potassium by rubidium." However, animals on such a diet are "hyperexcitable and develop tetany when exposed to the sound of a blast of air."

Heppel[21] studied the tissues of approximately 100 Gm. animals maintained for 45 days upon a potassium-low diet (0.01%). The serum potassium of such animals is about one-half the normal value and the chloride concentration is reduced about 15%. The muscles lose potassium and take up sodium in large quantities. In the muscles of normal rats most of the sodium is extracellular, but in the muscles of potassium-deprived rats most of it is intracellular. Potassium occurs almost entirely within the cells in both normal and depleted rats. Liver tissue of potassium-deprived rats shows no large changes in the electrolyte picture.

Growth of young rats is not possible on diets containing as little as 0.015% potassium.[18, 20] Apparently the minimum daily requirement for normal growth is about 15.0 mg. for the male and 8.0 mg. for the female.[17] As judged by potassium balance studies, the requirements of pregnancy and the greater requirements of lactation are supplied by diets containing, respectively, 0.14% and 0.503% potassium.[20]

SODIUM

Although a number of studies have been made of the sodium requirements of the rat, there is considerable variation in the reported findings. Osborne and Mendel[22] found that rats "grew with vigor" on a diet containing 0.035% of sodium and that "less than 0.04% of either sodium or chlorine in the food sufficed to permit these rats to complete their growth." Miller[23] obtained satisfactory growth of rats on a synthetic diet containing 0.07% sodium, but failed[24] to get normal growth from 0.03% sodium. Satisfactory growth and reproduction resulted when the sodium was increased to 0.46% of the diet.

Olson and St. John[25] found 0.23% of sodium in the diet of the rat to be insufficient, 0.53% to be the most satisfactory for growth and reproduction, and 0.785% or higher to be detrimental. Under the conditions of their experiments approximately 40 mg. of sodium per day is the optimum amount for the rat during the first 9 months after weaning. Mitchell and Carman[26] conclude that the addition of 1.0% of sodium chloride to a ration containing 0.047% of sodium and 0.041% chlorine enhances its growth promoting value. Schoorl[27] found that a diet containing 0.009% sodium limits the growth of rats during 10 weeks to a final body weight of about 60 Gm., but that a diet furnishing 0.2% sodium permits good growth. Kahlenberg, Black, and Forbes[28] report that rats on a diet containing 0.502% sodium, but with food intakes restricted to the amount consumed by their pair mates on a diet containing 0.007% sodium, increased in body weight from 51 Gm. to 116 Gm. during 70 days, while their pair mates grew at only about half this rate. They conclude that "sodium deficiency unfavorably affects the appetite, the increase in body weight, the storage of energy and the synthesis of fat and protein."

Orent-Keiles, Robinson and McCollum[29] prepared a diet containing 0.002% sodium which was still "distinctly superior to earlier diets as regards its vitamin content." Young rats placed upon this diet at weaning age show 100% mortality after 18 to 21 weeks despite the fact that their food intake is normal compared to control animals. The symptomatology comprises retarded growth and disturbances of the eyes and the reproductive function. Although keratinization of the corneal epithelium occurs, the authors conclude that the eye disturbances are different from those produced by vitamin-A deficiency. The sodium-depleted male remains potent for at least 75 to 96 days. In the female on this diet sexual maturity is delayed and the estrus rhythm and reproductive processes are affected. Only two of twelve sodium-depleted females became pregnant when mated to normal males, and these failed to deliver their young. Postmortem examination shows that practically all the tissues are affected. The bones of these rats are soft and easy to cut.

Both a chlorine-low diet and a sodium-and-chlorine-low diet lead to a symptomatology much different from that exhibited by animals on the sodium-low ration. Although animals on all three regimes exhibit retarded growth, sodium deprivation is the most detrimental and chlorine deficiency the least detrimental to growth. Both chlorine- and sodium-and-chlorine-depleted animals exhibit normal eyes and bones even after 90 days on these diets, in marked contrast with the eyes and bones of sodium-depleted animals.

Diets containing 0.07% sodium may be adequate for normal growth,[23] but about 0.5% sodium appears to be the most satisfactory for growth and reproduction of the rat.[24, 25]

CHLORINE

Marquis[30] reports that rats maintained for 140 days on a diet containing less than 0.01% chlorine grow much more slowly and attain a much lower final weight than controls on a diet containing 0.4% chlorine. Urinary excretion of chloride ceased, and after 123 days their blood contained 30% less and their organs 10 to 20% less chlorine than the controls.

Orent-Keiles, Robinson, and McCollum[31] have shown that both a chlorine-low (% not given) and a sodium-and-chlorine-low diet retard growth but not to the extent of sodium deprivation alone. The eyes and bones of rats on these diets are normal in contrast with the eye disturbance and soft bones resulting from sodium deficient diets.

Barelare and Richter[32] report a study, by the method of self-selection, of the sodium chloride requirement of pregnant rats. The average daily intake (17 pregnancies) in the 10 days before conception was 69 mg., in the first 10 days of gestation it was 147 mg., in the second 10 days of gestation it was 219 mg., and in the first 10 days post partum it fell to 105 mg. The significance of this increased sodium chloride appetite during pregnancy is not clear.

Miller[33] in a study of the sodium requirements of the rat observed that under the conditions of his experiment about 5 mg. of chlorine per day are sufficient to supply the requirements of growth, gestation and lactation (0.46% sodium in the diet).

IRON

The total amount of iron in the body of the rat is quite small (about 0.005% or less), and approximately two-thirds of this is present in the blood as an essential constituent of the hemoglobin of the red blood cells. Obviously, then, an iron-poor body tends to be anemic, and studies of the dietary requirements for iron are closely related to studies of anemia. Since the recognition of the fact that the presence of copper is necessary for the synthesis of iron into the hemoglobin molecule, it has been impossible to separate completely a discussion of the nutritive requirements for these two elements.

Miller, Forbes, and Smythe[34] report that the bodies of rats maintained for five weeks upon whole or skim milk powder diets contain about 50% (0.0022 to 0.0025%) of the normal amount of iron (0.005%). (Milk is deficient in iron and copper.)

Rose and Hubbell[35] have shown that young rats depleted by milk feeding to approximately 4 gm. of hemoglobin per 100 cc. of blood have an iron content of about 0.0013%. Similarly depleted animals were fed supplements of 0.00038 mg. of copper plus either 0.0019, 0.0038, or 0.0057 mg. of iron per gram of body weight per day until the hemoglobin had increased to about 14 Gm. per 100 cc. Depending upon the dosage of iron given the bodies of the males averaged between 0.0030 and 0.0034% of iron and the females averaged between 0.0034 and 0.0038% of iron. The females stored on the average about 12% more iron than the males when the intake was the same per gram of body weight.

It has been clearly demonstrated in the anemic rat[36, 37, 38] that when inorganic iron is fed in the absence of copper there is a decided increase in the total iron content of the liver and spleen but no appreciable increase in blood hemoglobin.

Elvehjem and co-workers obtain optimum hemoglobin regeneration in the milk-fed anemic rat from supplements of 0.5 mg. of iron and 0.05 mg. of copper per day. Hart, Elvehjem, and Kohler[39] present evidence to indicate that the available iron and copper are the only constituents of liver needed for maximum hemoglobin regeneration in rats on a milk diet.

Mitchell and Miller[40] conclude that 0.25 mg. of iron per day is adequate to maintain normal hemoglobin values for the rat if sufficient copper is present in the diet. With daily doses of less than 0.1 mg. of copper there was a slight but definite retardation of hemoglobin synthesis if the iron intake was near the minimum requirement.

Daniels and Everson[41] report excellent reproduction of rats through several generations on a diet of boiled Guernsey milk to which were added 0.8 mg. of copper, 5 mg. of iron, 2 drops of 2 per cent potassium iodide solution and 1.5 mg. of manganese per 100 cc.

Evans and Phillips[42] report that rats fed fresh milk mineralized with 1.0 mg. of iron, 0.1 mg. of copper and 0.1 mg. of manganese per 100 cc. "remained healthy and vigorous through five generations," although "reproduction difficulties were observed."

Alt[43] reports that young, from mothers receiving a stock diet, contained an average of 0.254 mg. of iron per rat at birth. First litter offspring from mothers receiving a whole milk diet contained only 0.11 to 0.15 mg. of iron and second litters contained only about 0.07 mg. of iron. First litter offspring had normal hemoglobin values at birth and did not show pathologic anemia during the suckling period. Second litter offspring showed about one-half the normal hemoglobin content at birth and rarely survived longer than ten to twelve days. A single pregnancy did not cause anemia in the female rat but resulted in marked depletion of the iron content of the liver. A second pregnancy resulted in moderate anemia. Supplements of 5.0 mg. of iron per day protected both the mother and the offspring against iron deficiency.

Underwood[44] concludes that inorganic ferrous and ferric iron at limited intakes are equally effective in the anemic rat in inducing growth, hemoglobin regeneration and storage of iron in the liver.

Elvehjem, Hart, and Sherman[45, 46, 47] and Kohler, Elvehjem, and Hart[48] have shown that it is impossible to evaluate foods as a source of iron in the diet on the basis of their total iron content. They conclude that the ionizable, or non-hematin, iron which they determine with the αα, dipyridyl reagent, is identical with the available iron in food. According to their figures for a number of different foods the available iron may vary from 20 to 90% of the total iron present. Although other workers have reported certain discrepancies between the values obtained by bio-assay and by the dipyridyl method, there seems to be fair agreement between the two methods.[49, 50, 51]

There is ample evidence[40, 52, 53, 54, 55] that the young anemic rat can regenerate hemoglobin at the rate of about 10 Gm. in 6 weeks when a readily available total of about 0.25 mg. of iron and 0.05 mg. of copper is furnished per day in a supplemented milk diet. The greater requirements for reproduction and lactation are adequately supplied by 5 mg. of iron and 0.5 mg.

of copper per day, [41, 43] but these amounts may be considerably more than the minimum requirement.

COPPER

Numerous workers have shown that animals restricted to a milk diet develop a nutritional anemia. Hart, Steenbock, Waddell, and Elvehjem[56] reported the first experiment recorded in the literature giving to copper in association with iron the specific function of hemoglobin formation on an otherwise satisfactory diet. They added 0.25 mg. copper as $CuSO_4$ and 0.5 mg. iron as $FeCl_3$ per day to the whole milk diet of anemic rats. The hemoglobin in the blood increased from 2.7 Gm. to 13.3 Gm. per 100 cc. in a period of 6 weeks. They found 0.01 mg. copper daily to be deficient and 0.06 mg. copper to be corrective. Despite some early conflicting reports these findings have been repeatedly confirmed in many other laboratories. There is no doubt that milk is deficient only in iron and copper as far as hemoglobin formation in the rat is concerned. Underhill, Orten, Mugrage, and Lewis[57] found a normal blood picture in rats continued on a milk, iron and copper ration for 667 days.

Josephs[58] concludes that "copper has no effect upon iron retention" but the absence of copper prevents the utilization of iron for hemoglobin formation. Elvehjem and Sherman[59] report that when inorganic iron is fed in the absence of copper the hemoglobin content of the blood remains unchanged but the amount of iron stored in the liver and spleen is proportional to the amount of iron fed. In the presence of copper, the rate of hemoglobin formation is dependent upon the iron intake.

Stein and Lewis[60] conclude that copper has two distinct roles in blood formation: first, its well recognized catalytic action on hemoglobin formation, and second, a stimulating effect on erythropoiesis. They report that copper alone given as a supplement to the milk diet of anemic rats shows a definite erythropoietic action without any influence on hemoglobin formation.

Mitchell and Miller[61] report that rats receiving less than 0.1 mg. of copper per day show a slight but definite retardation of hemoglobin synthesis if the iron intake is near the minimum requirement.

Cohen and Elvehjem[62] found the A component of cytochrome to be absent or greatly reduced in heart and liver tissue taken from severely anemic rats. Iron alone had no effect on the regeneration of this compound, but the feeding of copper alone or copper plus iron to the anemic rats for a few days greatly intensified the spectrum of the A component. They found also that the amount of oxidase in the liver of anemic rats as determined by the "nadi" reagent was greatly reduced. The feeding of copper or copper and iron markedly increased the oxidase content, but iron alone had no effect. Thus very definite changes aside from retarded hemoglobin formation can be observed in animals receiving insufficient copper.

Keil and Nelson[63] by the administration of copper alone to anemic rats demonstrated a lowering of the peak of their glucose tolerance curves indicating "an acceleration of glycogen formation and thus a more rapid removal of glucose from the blood."

Lindow, Peterson, and Steenbock[64] report that the absolute amount of copper in the body of a rat on an adequate ration increases from 0.0108 mg. at birth to 0.4422 mg. at about 200 days of age. The daily addition of 5 mg. of copper to the normal ration of rats increases the copper content of the skeleton, the kidney, the spleen, and the liver 1.6, 2, 5, and 20 times, respectively, over the normal amount, and increases the blood copper from 0.00543 to 0.0821 mg. per 100 cc. The total copper content of rats on an anemia-producing diet was the same at 3 weeks of age and at the time of death (9 to 11 weeks).

Daniels and Everson[65] obtained satisfactory reproduction of rats receiving mineralized milk containing 0.8 mg. added copper per 100 cc. Evans and Phillips[66] report reproduction of rats through five generations upon mineralized fresh milk containing as little as 0.1 mg. of added copper per 100 cc. Cunningham[67] reports good growth and reproduction on diets providing as little as 0.011 mg. copper, or as much as 7.5 mg. of copper per day. The average copper content at birth of the young from females on the low copper diet was .0045 mg. and 0.0146 mg. from those on the high copper diet. The author indicates that at least within these limits the amount of copper in the diet has no effect on the growth and reproduction of rats.

Boyden, Potter, and Elvehjem[68] have shown that, during *ad libitum* feeding of diets containing 0, 500, 1000, 2000 and 4000 p.p.m. of added copper, rats ingested voluntarily 5.05 to 11.8 mg. of copper daily. At 4000 p.p.m. the food intake was so restricted as to result in partial starvation and death within one week. Slight toxicity was observed on 500 p.p.m. with increasing toxicity on higher levels as indicated by growth records. Possibly differences in the basal diet may account for the lack of agreement concerning the amount of copper which is toxic.

Schultze, Elvehjem, and Hart[69] found the copper of copper caseinate, glycine amide biuret, alanine amide biuret, hemocyanin and whole wheat to be readily utilized by severely anemic rats but copper hematoporphyrin was not utilized even when fed at high levels. How much of the copper in natural foods, if any, occurs as copper porphyrin or in some other non-available form is not known.

Diets furnishing less than 0.05 mg. of copper per day certainly are very little, if any, in excess of the minimum requirement of the rat for growth and reproduction and may possibly be considerably below the optimum requirement.

IODINE

In the absence of adequate amounts of iodine in the diet a condition known as goiter develops. Levine, Remington, and von Kolnitz[70] have prepared a diet which contains only about 15 micrograms of iodine per kilogram. Young rats fed this ration consume about 0.15 microgram of iodine per day and develop severe goiter within 5 weeks; yet the addition of 3 micrograms per day of iodine as potassium iodide effectively prevents its development. The average fresh thyroid weight (per 100 Gm. body weight) of such animals is about 53 mg., in contrast with 12 mg. for rats receiving added iodine, its dry matter content is 19% against 28%, and its iodine content, on a dry basis, is 0.008% against 0.267%. Using as a criterion "the smallest amount of iodine necessary to prevent any significant thyroid enlargement," they conclude that the minimum iodine requirement is about 1 to 2 micrograms per rat per day.

Remington, Remington, and Welch[71] conclude that during the period of rapid growth (50 to 150 Gm.) the *normal* thyroid weighs 10 mg. per 100 Gm. of body weight for both sexes. Thereafter this value declines to about 7.5 mg. for full-grown rats. The average dry matter content is 33%; the average iodine content is 0.20% of the dried gland, and there are no significant variations attributable to age or sex. Remington and Remington[72] find that 265 micrograms of iodine per kilogram in an otherwise well-balanced diet is sufficient to protect growing rats from iodine deficiency. Growth and the utilization of food are not affected by amounts of iodine as high as 50 to 100 times the minimal protective dose. If iodine-enriched diets are fed to rats with enlarged and hyperplastic thyroids, the gland tends to return to normal weight and dry matter content, but such glands can store more iodine than normal ones.

Remington and Levine[73] conclude that the degree of goiter produced in the rat is not affected by varying the calcium content of the ration, the Ca:P ratio, or by the presence or absence of vitamin D. Remington, Coulson, and Levine[74] find that the antigoitrogenic properties of milk, oysters, and haddock are in proportion to their iodine content, and of the same order of magnitude as those of potassium iodide.

A minimum of about 1 to 2 micrograms of iodine per rat per day are sufficient to prevent any significant thyroid enlargement. Well-balanced diets containing about 265 micrograms of iodine per kilogram protect growing rats from iodine deficiency.

MAGNESIUM

Kruse, Orent, and McCollum[75, 76] report that magnesium deficiency produced by a diet containing only 1.8 parts per million of magnesium is characterized by "vasodilatation, hyperirritability of the nervous system, cardiac arrhythmia, spasticity and fatal tonic clonic convulsions."

Tufts and Greenberg[77, 78, 79, 80] have published an extensive study of magnesium deficiency in rats. They report two phases of the deficiency: first, vasodilatation, hyperemia and hyperexcitability and second, malnutrition, cachexia, and kidney damage. At a level of 0.4 mg. of magnesium per 100 Gm. of food all their animals show vasodilatation in 7 to 9 days and die from spontaneous convulsions in from 21 to 30 days. Increasing the magnesium content of the diet decreases the number of animals showing these symptoms until at 5 mg.% only about one-quarter of the total number of animals show hyperexcitability after two or three weeks on the diet. If the calcium level is increased from 0.87% to 1.16% of the diet a much more severe vasodilatation occurs and a much greater percentage of the animals show convulsions despite the presence of 5 mg.% of magnesium in both diets.

With diets of liberal normal calcium content (0.87%) 5 mg.% magnesium is the borderline amount necessary for good growth. Females reared on this diet give birth to young of normal weight and normal magnesium content. These young rats while suckling, however, develop all the symptoms of deficiency at the age of 2 or 3 weeks. Furthermore, the percentage of magnesium does not increase in their bodies as it does in young suckled by mothers on a normal diet, from which the authors conclude that the mother's milk is deficient in magnesium. Females reared upon a diet containing 1.16% calcium and 5 mg.% magnesium were in "too poor a condition to breed." Many pregnancies were evident in a group of females fed a diet containing 1.66% calcium and 13 mg.% magnesium but in all cases the litters were either resorbed, or, if born, were eaten by the mothers immediately upon birth.

Extensive renal damage is observed in animals fed a diet containing 2.0 mg.% magnesium and 0.87% calcium for fifteen weeks and likewise in rats on a diet containing 5 mg.% magnesium and 1.16% calcium. The pathologic changes in the kidneys include calcification in the cortico-medullary zone and pyramids and at a later stage in the cortex. In earlier stages degenerative changes in the tubules are a more constant finding than calcification. Magnesium deficiency produces an increase in the volume of urine and in the excretion of protein, but no blood or casts are present. The blood serum protein content gradually decreases until it reaches levels at which edema develops. Calcium is increased in heart and muscle from 50 to 100% and in kidneys as much as 15 times.

Cunningham and Cunningham[81] report that bladder calculi are formed in 30 to 100% of their rats on a diet containing 2.5 to 6.0% calcium carbonate and 0.017% magnesium. The addition of either 0.76% magnesium carbonate or 2.0% sodium acid phosphate to the ration prevented the formation of calculi.

Watchorn and McCance[82] report that rats maintained on a diet containing approximately 4.0 mg.% magnesium develop diarrhea and melena beginning about the tenth day. This is followed by hyperemia of the skin, loss of hair and in some cases hyperpnea and nervousness. These symptoms disappear in 7 to 10 days and thereafter the rats appear normal until near the end of their experimental period (12 weeks). Postmortem examination of the animals revealed that the kidneys were calcified, the bones brittle and the teeth sometimes white or translucent. The magnesium content of the bones was reduced to approximately two-thirds and that of the teeth to one-half of the normal level. Within 14 days on the diet the blood magnesium was reduced to approximately one-half of its normal value. The magnesium content of the soft tissues was not significantly altered by this diet.

Tufts and Greenberg[78] conclude that "under otherwise optimum conditions a magnesium intake in the diet of approximately 4 mg. per kilo of body weight per day approaches the minimal magnesium requirement of the rat."

From the available data it seems evident that with diets containing average amounts of calcium there must be present a little more than 5 mg.% of magnesium in the diet to permit normal development and reproduction of the rat.

MANGANESE

Orent and McCollum[83, 84] report that young rats grow to maturity in an apparently normal manner on a "manganese-free diet." The females go through normal estrual cycles and when mated with potent males produce the normal number of young. However, they fail to suckle their young. Females raised on this "manganese-free diet" plus 0.005% manganese are able to wean successfully a large number of their offspring. Third generation animals were obtained and apparently these could have been continued. It appears that "0.005% manganese is near the minimal amount" required for the support of normal reproduction and lactation. Male rats raised on this "manganese-free diet" show testicular degeneration resulting in complete sterility but otherwise appear normal. The addition of 0.005 to 0.05% of manganese in the form of the chloride will keep males in sexual potency for at least 14 months. Waddell[85] also reports total sterility of male rats upon a low manganese diet.

Daniels and Everson[86] conclude that "the high percentage of deaths of the young of animals reared on milk diets fortified with copper and iron is due to congenital debility, the result of too little manganese in the diet of the mother." Their milk-copper-iron diet furnished 0.022 mg. of manganese per day while animals receiving the same diet plus manganese and those on the stock diet ingested 1.202 mg. and 0.816 mg. per day, respectively. Of the young born to milk-manganese and stock-diet females nearly 100% were successfully weaned with excellent weaning weights. Of the young born to low-manganese females and suckled by normal mothers only about 30% were successfully weaned and these had much lower weaning weights, indicating a weakness of the young at birth. Animals reared from infancy on supplemented cow's milk containing 0.03 mg. of manganese per liter were able to suckle vigorous foster young successfully, but this was too little manganese for the development of normal young in utero.

According to McCarrison[87] rats receiving 0.015 mg. of manganese per day ($MnCl_2$ supplement) grow significantly more rapidly than their controls receiving only 0.006 mg. per day. A supplement of 0.56 mg. of manganese (as MnO_2) per day had a retarding effect upon growth which became evident only after 32 days consumption of the salt and continued until the end of the experiment at 135 days.

Becker and McCollum[88] fed a basal low-manganese-ration with supplements of between 5.0 and 100. mg. of manganese per day. (Testicular degeneration occurred in nearly all the males maintained on the basal diet.) "Reproduction was excellent" on all of the supplemented diets, but the one furnishing 100 mg. of manganese per day "showed some depression of growth." There were no other indications of toxicity even at this very high level of intake. The authors suggest that the high level of phosphorus in the diet (0.72%) prevents symptoms of toxicity by reducing the amount of absorbable manganese.

Skinner[89] reports that "female rats which receive 10 mg. of manganese per day above that in the stock ration (13.4 mg. per kilo) are as successful in rearing their young as females receiving the stock ration only."

Perla, and Perla and Sandberg[90, 91] report that they have "demonstrated a metabolic interdependence of manganese and vitamin B1." Supplementing an adequate normal diet with 400 micrograms of vitamin B1 daily resulted in the females exhibiting cannibalism, loss of maternal instinct, interference with lactation and progressive loss of fertility. The addition of 2 mg. of manganese per day per rat to this diet completely neutralized the unfavorable effects of the excess of vitamin B1. Animals receiving the unsupplemented diet plus 2 mg. of manganese per day likewise showed a disturbance in lactation, cannibalism, and a loss in maternal instinct. In spite of this fact the animals receiving an excess of both vitamin B1 and manganese reared their young normally. More recently these authors report[92] that the addition of 1 mg. of manganese daily to a vitamin-B1-deficient diet shortens by a week or ten days the average depletion period and the time at which the blood pyruvic acid rises in the rat.

The differences in response to extra dietary manganese obtained by various workers[88, 89, 90, 91] would seem to indicate that its metabolism may be related to more than one other dietary factor. It may be necessary to reinvestigate the role of manganese in the light of the interesting papers of Perla and Sandberg. However, with diets of average vitamin B1 content the following conclusions seem justified. Failure in lactation may occur in animals raised on "manganese-free diets" as suggested by Orent and McCollum,[84] but adequate lactation is possible on supplemented cow's milk containing 0.03 mg. of manganese per liter.[86] It seems that 0.02 mg. is insufficient, about 0.5 is minimal and 0.8 mg. of manganese per day is adequate for excellent reproduction.

ZINC

Todd, Elvehjem, and Hart[93] and Stirn, Elvehjem, and Hart[94] have shown that the growth of rats on a synthetic diet low in zinc is markedly retarded as compared to that of control animals receiving the same diet with added zinc. Hove, Elvehjem, and Hart[95] report an average daily growth over a six week period of less than 1.5 Gm. for rats on a zinc-low ration and over 3.0 Gm. for litter mates on the same ration supplemented with 300 micrograms of zinc per day. The average daily zinc ingestion of the zinc-deficient rats was 22 micrograms. It was found that 30 micrograms per day decreased the zinc deficiency and 40 micrograms entirely prevented it. Evidence is presented indicating a "marked delay in intestinal absorption in zinc-deficient rats."

The same authors further report[96] that young rats receiving about 7 micrograms of zinc per day show only slightly less zinc in the whole carcass (51 to 57 mg. per kilo dry weight) than their controls which received 100 micrograms daily (65 to 77 mg. per kilo dry weight). Bone, teeth, and blood show the greatest reduction. Greatly retarded growth rate is the only definite symptom which has been observed in this severe zinc deficiency. As yet there is no indication of what the specific function of zinc may be in the living cell.

Although the pancreatic enzymes (especially the proteinase and amylase) show a lessened activity in the zinc-deficient rat this is interpreted as a secondary effect related to a general metabolic debility.

Sutton and Nelson[97] report that the limit of tolerance of young rats to zinc lies between 0.5 and 1.0% of the diet. A concentration of 0.1% has no appreciable effect upon growth, reproduction or the blood picture; yet 0.5% affects both reproduction and the blood picture. In the first pregnancy most and in the second all the young, although fully developed, are born dead. At a 1% level no reproduction occurs. Hemoglobin values are somewhat lower than normal in the animals receiving 0.5% of zinc, but the erythrocyte values are normal. Both hemoglobin and erythrocyte counts are very low in animals on the 1% level, but they return to normal after withdrawal of the zinc from the diet.

An average daily ingestion of 7, 22, or even 30 micrograms of zinc is insufficient, yet 40 micrograms per day appear to be adequate for normal growth of the rat.[95, 96]

COBALT

Many workers[98, 99, 100, 101, 102, 103] have demonstrated that cobalt produces a marked polycythemia when administered in small amounts to rats. Orten, Underhill, Mugrage, and Lewis[100, 101] conclude that 0.5 mg. of cobalt when fed daily to rats on a milk-iron diet produces an actual increase in the number of circulating red blood cells. This polycythemia persists as long as the cobalt supplement is continued. Orten[103] has shown that the administration of cobalt produces significant increases in the proportion of reticulocytes in the blood preceding the typical elevation in the concentration of pigment and erythrocytes. These results indicate "an increase in the rate of formation of hemoglobin and erythrocytes and that a passive accumulation of red cells as a result of a diminished rate of cell destruction does not occur." The growth rate of the cobalt-fed animals was distinctly lower than that of the controls.

Stare and Elvehjem[101] report that the entire body of rats fed a milk, iron, copper and manganese diet contains *less* than 0.01 mg. of cobalt. The presence of 0.04 to 0.05 mg. of cobalt in the entire body of a rat is sufficient to produce a decided polycythemia. Young rats receiving more than 0.6 mg. of cobalt per day show definite toxic effects. At levels of 1.0 and 1.5 mg. per day rats rapidly lose weight and at 2.0 mg. per day they survive for only about two weeks. With older animals no toxic effects are observed at levels of less than 1.0 mg. per day.

Underwood and Elvehjem[104] found the mean cobalt content of nine litters of new born rats to be 0.15 part per million and that of seven rats at weaning age to be 0.14 part per million of the dry matter. Samples of milk were found to contain from 8 to 18 micrograms of cobalt per liter, with a mean of 11 micrograms. Rats consuming sufficient milk to supply them with about 0.6 microgram of cobalt per day grew and regenerated hemoglobin normally and remained healthy for as long as 9 months. Later, Underwood[105] found that addition of cobalt to a highly purified synthetic ration supplying only 0.4 microgram cobalt daily brought about no improvement in growth or hemoglobin production over a period of 6 weeks. In view of the known requirement of sheep and cattle for cobalt[106, 107, 108] it seems probable that rats may require cobalt, but apparently this need, if it exists, is less than 0.4 microgram per day.

APPARENTLY NON-ESSENTIAL INORGANIC CONSTITUENTS

Aluminum. McCollum, Rask, and Becker[109] found that 600 parts per million of aluminum in the diet of the rat was not deleterious to growth, reproduction, or general well being. Myers and Mull[110] reared four generations of rats and weaned a fifth on diets furnishing 2 mg. of aluminum per rat per day with no signs of inhibited growth or reproduction. Hove, Elvehjem, and Hart[111] conclude that if aluminum is needed by the rat during growth and reproduction one microgram per day will satisfy this requirement.

Arsenic. Hove, Elvehjem, and Hart[112] conclude that if arsenic is essential for the growth and development of rats or for the building of hemoglobin or red blood cells, 2 micrograms per day are sufficient to satisfy this requirement. No positive results on growth stimulation of experimental animals by feeding small amounts of arsenic have been recorded in the literature.

Boron. Hove, Elvehjem, and Hart,[113] in the only study on record of the role of boron in animal nutrition, conclude that if boron is needed by the growing rat, 0.8 microgram per day satisfies this requirement.

Bromine. Winnek and Smith[114] prepared a synthetic diet which supports growth in the rat and has a bromine content of less than 5 parts per 10 million. Females on this diet both with and without added bromine had litters but did not successfully raise their young. No evidence was obtained to indicate that bromine is essential in the nutrition of the rat.

Fluorine. The constant presence of fluorine in the bones, teeth and other tissues of animals and man has led to the general view that fluorine is an essential element in nutrition. However, all attempts to demonstrate an impairment of growth or health from diets containing minimal amounts of fluorine have failed. Sharpless and McCollum[115] conclude that fluorine has no essential physiologic function. Phillips, Hart, and Bohstedt[116] conclude "that fluorine in concentrations greater than 1 part in 10,000,000 in the diet has no essential function in the metabolism of the rat." Evans and Phillips[117] found mineralized milk containing 0.1 to 0.2 part per million of fluorine to be a "good diet through five generations of rats." Additional fluorine from 0.1 to 20.0 parts per million caused no measurable improvement in the rat. De Eds and Thomas[118] and also Smith and Leverton[119] find that about 14 parts of fluorine per million of diet is sufficient to produce chronic intoxication as indicated by bleaching of the rat incisors.

Silicon. King and Belt[120] in their review of "The Physiological and Pathological Aspects of Silica" state that "the almost universal distribution of silica makes it probable that silicon will be at least a likely contaminant, if not an essential ingredient, of all protoplasm." However, the writer knows of no indication of a nutritional need for this element.

AMINO ACIDS AND PROTEINS

Proteins are required by the animal body, mainly, for the replacement of the tissue proteins, which are broken down in the normal metabolic processes, and for the building of new tissues in growth and reproduction. Proteins digested and absorbed in excess of these requirements are for the most part used for the production of energy—either stored as carbohydrate or fat, or expended in work or heat.

Proteins are made up of at least twenty-two different amino acids. The amounts and proportions of these acids vary tremendously in proteins from different sources. Inasmuch as all of the generally recognized amino acids occur as components of tissue proteins, obviously each must be made available, either preformed in the diet or by synthesis in the organism from other materials. The recognition of this fact naturally raises the question as to the nutritional importance of the individual amino acids.

Amino Acids.* Until comparatively recently only three of these compounds, namely, tryptophane, lysine, and histidine, had been shown definitely to be indispensable components of the diet. In 1931 Rose[124] reported investigations using diets in which the proteins were replaced entirely by mixtures of highly purified amino acids. From this work it was apparent that the known amino acids, when incorporated in otherwise adequate diets, were incapable of supporting growth. This led eventually to the isolation and identification, by McCoy, Meyer, and Rose,[125] of a new indispensable dietary component, now known as threonine. Feeding experiments with this new amino acid incorporated in the otherwise adequate diet constituted the first successful effort to induce growth in animals upon diets carrying synthetic mixtures of highly purified amino acids in place of proteins. Following this discovery, the importance of the individual amino acids was determined by omitting them from the food one or more at a time. (An indispensable dietary component has been defined, for the present at least, as one "which cannot be synthesized

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* From reviews by Professor William C. Rose concerning the "Nutritive Significance of the Amino Acids" where a complete bibliography may be found.[121, 122, 123]

TABLE IV

Final Classification of the Amino Acids with Respect to Their Growth Effects

Essential	Non-essential
Lysine	Glycine
Tryptophane	Alanine
Histidine	Serine
Phenylalanine	Norleucine
Leucine	Aspartic acid
Isoleucine	Glutamic acid
Threonine	Hydroxyglutamic acid
Methionine	Proline
Valine	Hydroxyproline
*Arginine	Citrulline
	Tyrosine
	Cystine

**Arginine can be synthesized by the rat, but not at a sufficiently rapid rate to meet the demands of normal growth.*

by the animal organism, out of the materials ordinarily available at a speed commensurate with the demands for normal growth."[122]) The results of these experiments are summarized in Table IV.

As a more severe test of the correctness of the above classification, experiments were conducted in which animals received a diet devoid of all the non-essential acids. Under such extraordinary conditions necessitating the synthesis of twelve tissue components simultaneously, a failure in growth would not have been surprising. Excellent growth occurred, thereby providing the final proof that only ten of the twenty-two amino acids of proteins need be included preformed in the diet of the rat.

TABLE V

Minimum Amount of Each Essential Amino Acid Necessary to Support Normal Growth in the Rat When the Non-essentials Are Included in the Food

Amino acid	Per cent
Lysine	1.0
Tryptophane	0.2
Histidine	0.4
Phenylalanine	0.7
Leucine	0.9
Isoleucine	0.5
Threonine	0.6
Methionine	0.6
Valine	0.7
Arginine	0.2
	5.8

Experiments were also undertaken to determine how much of each indispensable amino acid is necessary to induce optimum growth. This was accomplished by varying the proportions of one while all the others were maintained at constant levels. The results are summarized in Table V and are to be regarded as "strictly tentative."

Preliminary experiments indicate that reproduction is possible on a diet in which the protein has been replaced by amino acids, but no data are available concerning the specific requirements.[123]

Proteins. Although a number of proteins are lacking in certain of the essential amino acids (gliadin of wheat is deficient in lysine and zein of corn is practically devoid of lysine and tryptophane), the feeding of a mixture of proteins of plant and animal origin will usually insure the presence of all the necessary ones. Two proteins, each of which is deficient in one or more of these essential components, may mutually supplement each other to provide an adequate source of nitrogen.

Now that the amino-acid requirements for growth have been established, there still remains the question of the optimum protein intake for all the physiologic needs of the organism. Hogan and Pilcher,[126] using rations varying from 7 to 33% protein, find that rats on the higher concentrations make better gains than those on the lower levels. Hogan, Johnson, and Ashworth[127, 128] report that rats on a high protein ration (26.2%) make greater gains and store more water, protein, and energy, but less fat, than do their pair mates on a low protein food (10.0%). Forbes, Swift, Black, and Kahlenberg[129] describe studies of the effects of four different levels of dietary protein (10, 15, 20, and 25%) when equicaloric amounts of the rations are fed. With increased protein intake there is an increase in total gains, an increase in protein stored and usually a decrease in fat gained per unit of protein stored. Hamilton[130] concludes that the growth-promoting value of a diet is increased as the protein of the ration is increased from 4 to 16%, is unchanged between 16 and 30%, and is decreased when the concentration of protein is above 30% of the food. Diets containing less than 16% protein produce gains having more fat but less protein than is produced on a well-balanced ration. Gains of approximately the same composition are produced by foods containing between 16 and 42% protein.

McCoy[131] has studied the growth, reproduction, lactation, and body composition of animals receiving different levels of protein in the diet. Vitamin-free, fat-free, salt-low casein was substituted for a part of the dextrin of the basal ration to produce foods containing about 15, 25, and 40% protein. In *ad libitum* feeding experiments animals receiving the high- and medium-protein diets grow at about the same rate, yet distinctly more rapidly than those receiving the low-protein ration. In paired feeding experiments the rate of growth and the percentage of nitrogen of the animal parallels the protein intake. The percentage of fat in animals on the 40% diet is consistently lower than in those from either the 25 or 15% rations. In reproduction studies continued through five generations, it has been found that females receiving the high protein diet produce more litters and more young per litter than those receiving either of the other foods. The medium-protein ration produces the most satisfactory lactation, as judged by the total weight of the litter and individual weights at weaning age (21 days). Although the low-protein diet permits reasonably satisfactory growth, reproduction and lactation, it is distinctly inferior to either of the other foods by all methods of comparison.

Optimum growth of the rat occurs on diets containing between 25 and 30% protein; and, although excellent reproduction also occurs on 25% protein diets, even better reproduction appears to be possible on rations containing 40% protein.

FATTY ACIDS

In 1929 Burr and Burr[132] reported a new deficiency disease of the rat produced by the exclusion of fats from the diet. On such a ration animals grow for a time, but soon develop deficiency symptoms: the skin becomes scaly; the end of the tail appears inflamed and swollen, and later becomes heavily scaled, ridged, and necrotic. The hair on the back of the body becomes filled with dandruff, and around the face and neck it tends to fall out. Hemorrhagic spots and sores appear on the skin. Ovulation is irregular in the females; the males usually fail to mate; hematuria, albuminuria, and kidney lesions appear regularly.[133]

Evans, Lepkovsky, and Murphy[134] conclude that successful gestation is not possible in rats kept on a fat-free diet. In about one-fifth of the pregnant animals fetal resorption occurs; in others the gestation period is prolonged and there is frequent maternal mortality. Small litters of undersized young are born dead, or so weak that they die soon after birth. Lactation is also impaired. Gestation is still unsuccessful when large amounts of saturated fatty acids are added to the diet, but becomes satisfactory upon the further addition of unsaturated fatty acids. Males on fat-free diets invariably become sterile. The daily addition of 50 mg. of a preparation of unsaturated fatty acids from corn oil can cure as well as prevent their sterility.

Burr, Burr, and Miller[135] conclude that both linolenic and linoleic acid are effective and are of about equal value in curing rats suffering from a fat deficiency, whereas oleic acid is ineffective. Martin[136] has tentatively placed the minimum level of methyl linoleate for optimal growth effects at 30 mg. per rat per day. Turpeinen[137] obtains a maximum growth response in plateaued fat-deficient females when approximately 100 mg. of methyl linoleate is fed daily, and a similar response from only 33 mg. of the methyl ester of arachidonic acid. Hume, Nunn, Smedley-Maclean, and Smith[138] find linoleic acid to be about six times as potent as linolenic acid in promoting weight increases and healing skin symptoms of fat-deficient rats. The methyl ester of docosahexaenoic acid is also active in promoting growth, but it has little effect in curing skin lesions. Nunn and Smedley-Maclean[139] report that arachidonic acid is absent from the livers of rats fed for several months on a fat-deficient diet, but is present in those which, after a similar depletion period, receive 14 mg. methyl linoleate daily for 5 weeks. The authors suggest that linoleic and linolenic acids are the building stones essential for the production of more highly unsaturated acids.

Mackenzie, Mackenzie, and McCollum[140] have prepared a diet lower in fat than any previously reported. The daily consumption of lipid, based on the average food intake of 10 Gm. per rat per day, is approximately 27 mg., of which 25 mg. is methyl linoleate. The growth of animals receiving this diet for as long as 11 months compares favorably with those on a stock ration. Gestation is satisfactory and the young are normal in appearance but many of them die during the first 3 days. Results obtained by exchanging the young of the low-fat diet mothers with those of stock-diet females suggest that lactation on the low-fat ration is inadequate.

Growth and gestation appear to be normal in animals receiving 25 mg. of methyl linoleate per day, but lactation is unsatisfactory unless significant amounts of fat are also present in the diet.

VITAMIN A

Vitamin A is known to be essential for growth, normal vision, and the maintenance of various specialized epithelial surfaces of the body. In vitamin-A deficiency keratinization of epithelial tissues occurs extensively in the respiratory tract (including the lungs), the alimentary tract, the urino-genital tract, salivary glands, and ductless glands.

Fridericia and Holm,[141] Holm,[142] Tansley[143] and others have shown that rats maintained on vitamin-A-deficient diets develop "night blindness" as one of the early symptoms of the deficiency.

Renal and cystic calculi occur frequently in vitamin-A deficiency but, according to Clausen,[144] "the majority of investigators fail to find that a deficiency of vitamin A alone will cause stones." Many of the calculi-producing diets have been unbalanced in their mineral content and deficient in other vitamins.

The vaginal mucous membrane becomes permanently cornified in female rats deprived of vitamin A,[145] but estrus and ovulation continue to occur fairly frequently. This injury may be sufficient to prevent the fertilization and implantation of the ovum. Vitamin-A deficiency in the male leads to degeneration of the germinal epithelium and complete sterility.

Clausen[144] concludes that no justification exists for calling vitamin A "the anti-infective vitamin." Alterations in the mucous membranes of the respiratory tract are the chief cause of spontaneous infections during "A" deficiency.

Wolbach and Howe[146] and Orten, Burn, and Smith[147] have shown that both chronic and complete deficiency of vitamin A produce, upon the incisor teeth, "a loss of the normal orange pigment, the development of opacity, a distortion of shape, and eventual exfoliation of the erupted portions." Mellanby[148] reports that the teeth of young rats reared by mothers kept for several months on a diet low in vitamin A are deformed and pale in color (see Chapter 6 for more detailed discussion of the effects of this deficiency).

In 1919 Steenbock[149] suggested that vitamin-A activity was related to the yellow coloring matter of certain plants. In 1928 Euler[150] found that the daily administration of only 5 micrograms of pure carotene was sufficient to restore promptly the growth of rats suffering from vitamin-A deficiency. Moore[151] demonstrated the conversion of carotene into vitamin A in the animal organism by feeding it to depleted rats and showing that vitamin A then appears in the liver. Carotene was found to be effective at a level of 4 micrograms per rat per day.

Baumann, Riising, and Steenbock[152] report that the minimum daily dose of vitamin A necessary to produce storage in the liver is "between 25 and 50 blue units." When 3 blue units of a standard cod liver oil were fed daily to depleted rats growth was restored and ophthalmia was cured: with 1.5 or 0.75 blue units daily ophthalmia was not cured. (Three blue units are equivalent to approximately 1 International Unit of vitamin A.)

Goss and Guilbert[153] conclude that the minimum level to prevent vaginal cornification in the rat is 3.8 to 4.6 micrograms of vitamin A or 15 to 20 micrograms of carotene per kilogram of body weight per day. No detectable storage occurred until a level of 80 micrograms of carotene per kilogram of body weight was given. Guilbert, Miller, and Hughes[154] have shown that cattle, sheep, and swine require approximately 6 to 8 micrograms of vitamin A or 25 to 30 micrograms of carotene per kilogram of body weight, indicating that on a weight basis the requirements of a number of animals are about the same. This suggests that vitamin A is intimately concerned with the general metabolic activities of the body as a whole.

Cannon[155] reports that female rats depleted until they show xerophthalmia or weight loss generally do not mate. Those receiving an A-low diet plus 40 micrograms of carotene three times weekly mated and gave birth to living young in every instance.

THIAMIN (VITAMIN B_1)

It is well known that in experimental animals suffering from thiamin deficiency abnormal quantities of pyruvic and lactic acid accumulate in various tissues and body fluids. Lohmann and Schuster[156] made the fundamental discovery that cocarboxylase is a pyrophosphoric acid ester of thiamin, thus demonstrating the physiologic role of this factor in carbohydrate metabolism. Banerji and Harris[157] found that the excretion of bisulfite-binding substances (largely pyruvic acid) is increased with increasing degrees of thiamin deficiency.

Engel and Phillips[158] report that uncomplicated B_1 deficiency is *not* accompanied by peripheral nerve degeneration, as had been indicated in many of the early papers on this subject. Lipschitz, Potter, and Elvehjem[159] conclude that B_1 deficiency produces a general disturbance of carbohydrate metabolism rather than a condition characterized by lesions in the nervous system.

The early observation that fat exerts a "sparing" action on thiamin[160] is oxidation of carbohydrates, but not for the oxidation of fats. This is further now satisfactorily explained by the fact that thiamin is required for the confirmed by the observation of Arnold and Elvehjem[161] that, regardless of the amount of fat in the diet, the thiamin required by the dog is related directly to the non-fat calories of the diet. In addition, Stirn, Arnold, and Elvehjem[162] have shown that when the carbohydrate of a "thiamin-free ration" is isocalorically replaced by fat the resultant diet will alleviate the polyneuritic syndrome of thiamin-deficient rats and permit satisfactory growth.

Arnold and Elvehjem[163] report that rats receiving a ration containing 80 to 100 micrograms of thiamin per 100 grams of food grow at a normal rate. They conclude[161] that for most species of animals the thiamin requirement is approximately one microgram per gram of non-fat energy supplying food materials.

Sure[164] reports that 10 micrograms of thiamin per day are sufficient to cure polyneuritis and to permit continuous and satisfactory growth of the rat. However, 120 micrograms are required per day for lactation comparable to that obtained with a stock ration.[165]

RIBOFLAVIN

Riboflavin is now recognized as an essential constituent of the "yellow oxidation enzyme" present presumably in every living cell of all the higher forms of life. Riboflavin requirements, therefore, are closely related to body size and the mass of active tissue. Minimal requirements for various species have not been determined exactly because of experimental difficulties in obtaining basal diets deficient only in riboflavin.

Rats deficient in riboflavin develop a non-specific dermatitis on the ears, neck, chest, legs, and paws, which can readily be distinguished from the so-called "florid" dermatitis of vitamin-B_6 deficiency. Riboflavin deficiency stunts the growth of the young and causes a premature aging of the skin and the loss of hair.

Day, Langston, and O'Brien[166] and Day and Langston[167] observed almost 100% incidence of cataract in rats receiving a "vitamin-G"-deficient diet. Day, Darby, and Langston[168] present evidence that this deficiency, characterized by alopecia and cataract, can be prevented by the inclusion of riboflavin in the ration. More recently Day, Darby, and Cosgrove[169] report that the progress of cataract development of this type can be arrested in a high percentage of cases by the administration of riboflavin. They conclude that riboflavin is a specific cataract-preventive substance for the rat.

Bourne and Pyke[170] report that under conditions "identical" with those of Day and coworkers only 31% of their animals developed cataract. György[171] notes that in an extensive series of experiments with either partial or complete deficiency of various members of the B complex he has never observed cataracts. However, there seems little reason to doubt that, at least under some conditions, riboflavin deficiency produces cataracts in rats.

Day and Darby[172] have shown that the growth of rats is roughly proportional to the amount of riboflavin fed at levels of 30, 60, or 90 micrograms per week. Rats receiving 90 to 120 micrograms of riboflavin per week reached adult size and remained in apparent health for more than a year, although growth proceeded at a slightly subnormal rate.

Supplee, Bender and Jensen[173] report that, through a 2 to 10 microgram per day feeding range, one microgram of riboflavin per rat per day induces a growth response of about 1 gram per week for a period of 6 to 8 weeks. Booher[174] says that 3 micrograms of riboflavin per day as a supplement to the Bourquin-Sherman diet induces a weekly growth of about 3 grams.

According to Edgar, Macrae, and Vivanco[175] optimum growth is possible on a diet supplying about 40 micrograms of riboflavin per day. Sure[165] concludes that the rat requires 120 micrograms of riboflavin per day for the satisfactory rearing of young.

NICOTINIC ACID

There is still considerable uncertainty as to whether or not nicotinic acid is a dietary essential for the rat. Rhodes and Miller;[176] Birch, György, and Harris;[177] and Birch, Chick, and Martin[178] found that rats fed on pellagra-producing diets are able to thrive, whereas dogs or swine reared on a similar diet succumb. Since it has been shown that nicotinic acid is the substance missing from the pellagra-producing diet, it would be presumed that rats do not need this substance. Birch,[179] using a diet "absolutely free from nicotinic acid" but also deficient in other members of the vitamin B_2 complex, was unable during 150 days to observe any specific physiologic dysfunction which could be prevented by the addition of nicotinic acid to the diet. However, several workers have presented evidence that this substance is necessary for the rat. Von Euler and Malmberg[180] showed that the introduction of 1 mg. of nicotinic acid per day into a Sherman-Bourquin-like diet supplemented with a vitamin B_1 concentrate and 10 micrograms of flavin per day prolongs the life of rats. Funk and Funk[181] have reported (in abstract form only) that nicotinic acid and nicotinic acid amide have growth-promoting properties and food-sparing action for rats. György[182] reports that the addition of 0.1% of nicotinic acid to a diet upon which panmyelophthisis is a common occurrence completely prevents the appearance of these symptoms.

Frost and Elvehjem[183] have presented the most significant evidence that nicotinic acid or the amide are important factors in the nutrition of the rat. Using a basal ration containing dextrin and 12% of white corn, supplements of 1 mg. per day of either nicotinic acid or nicotinamide produce delayed yet marked growth stimulation in rats. However,[184] with another basal ration, which contains no corn, these compounds fail to enhance growth and animals so supplemented actually lose weight and die sooner than do controls. The authors suggest that "white corn supplies a factor necessary to the proper utilization of nicotinic acid by the rat."

Fouts and Helmer[185] find a supplementary action of nicotinic acid on the growth of rats maintained on the Goldberger ration with added riboflavin. However, without added riboflavin, nicotinic acid exercises an inhibitory effect on growth and raises the mortality above that of controls.

Macrae and Edgar;[186] Cook, Clarke and Light;[187] Dann and Subbarow;[188] and Emerson and Evans[186] under various experimental conditions have all failed to demonstrate any growth effects from either nicotinic acid or nicotinamide.

Harris and Raymond[189] report that appreciable quantities of nicotinic acid are excreted in the urine of rats even after they have been on presumably nicotinic-acid-free diets for long periods of time. "The most likely explanation seems to be that the rat can synthesize nicotinic acid under certain conditions."

Sure,[165] in a study of the requirements of the rat for lactation and growth, reports that "six litters have been successfully reared without nicotinic acid administration either to the mother or to the young." Very recently Axelrod, Madden, and Elvehjem[190] comment that "there is no clear cut evidence that nicotinic acid is required preformed in the diet of the rat."

PYRIDOXINE* OR VITAMIN B_6

The characteristic symptoms of pyridoxine deficiency consist of a typical acrodynia in which the "region about the nose becomes erythematous, denuded and eroded; the nose becomes moist owing to a nasal discharge; the paws become red, swollen and raw and in a few cases encrusted ears are observed."[191] Very little growth, if any, occurs on diets entirely devoid of this vitamin.[192, 193, 194, 195]

Lepkovsky,[193] who was the first investigator to isolate this factor in pure form, obtains optimal growth of rats with a daily dose of 10 micrograms of his new product. Dermatitis involving the feet, paws, ears, and areas around the mouth is promptly cured by this dosage of vitamin B_6.

Dimick and Schreffler[194] report that the survival period of rats deprived of pyridoxine rarely extends beyond 50 to 60 days; those sacrificed shortly before death show complete atrophy of the thymus. Supplements of 50, 20 or even 10 micrograms of B_6 per day given after the appearance of typical symptoms produce an immediate resumption of growth and complete healing of the dermatitis within 10 days. El-Sadr, Macrae, and Work[192] and Kuhn and Wendt[195] agree that about 10 micrograms of pyridoxine per day will meet the requirements of the rat for growth.

Sure[165] reports that, although 10 to 25 micrograms of B_6 per day are adequate for continuous growth, at least 50 micrograms are required for satisfactory lactation.

It has been known for some time that dermal abnormalities may arise from either an "essential fatty acid deficiency"[196, 197] or a pyridoxine deficiency.[171] The finding, by Birch and György,[198] that certain fats cure the dermatitis produced by B_6 deficiency suggests that some relationship exists between these two deficiencies. Birch[199] finds that a B_6 preparation will not cure rat acrodynia unless the fatty acids from corn oil are also fed. Quackenbush, Platz, and Steenbock[200] state that linoleic acid is curative for "essential fatty acid deficiency" and also for acrodynia. Schneider, Steenbock, and Platz[201] report that acrodynia can be cured by the so-called "essential fatty acids" (free from any B_6) or by a rice-bran concentrate. The curative action of this concentrate is dependent upon B_6 plus a second "accessory factor" (pyridoxine in crystalline form has only a temporary effect on acrodynia).

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* Name recommended by the Committee on Vitamin Nomenclature of the American Institute of Nutrition.

Salmon[202] reports that on a fat-free diet supplemented with carotene, calciferol, thiamin, riboflavin, and "factor 2" rats make very little growth and consistently develop erythema on the nose and feet, a generalized scaliness and fissuring of the skin and a severe hematuria. The addition of synthetic B_6 produces a marked stimulation of growth, prevents the erythema and reduces the severity of the scaly condition of the skin, but does not prevent the hematuria and the rats eventually succumb. The addition of methyl linoleate or corn oil (without B_6) prevents the hematuria as well as the erythema and scaliness of the skin, but produces relatively little growth. The further addition of B_6 results in a marked increase in the rate of growth. Thus, there seems to be very good evidence that vitamin B_6 and the essential fatty acids can to a certain extent mutually alleviate the deficiency of each other as far as the cure of dermal abnormalities is concerned.

Halliday[203] reports that pyridoxine deficiency in the rat produces a significantly heavier liver containing a higher percentage of total fatty acids. Even massive doses of choline fail to bring the liver weight and fatty acid content quite to normal.

Emerson and Evans[204] report "uniformly defective sexual behavior" in vitamin B_6-deficient rats. Antopol and Schotland[205] note the occurrence of "foci of muscle atrophy in vitamin B_6-deficient rats."

Apparently 10 micrograms of pyridoxine per day are sufficient for normal growth of the rat, but at least 50 micrograms are required per day for satisfactory lactation.

OTHER FACTORS

In addition to thiamin, riboflavin, and pyridoxine the rat requires one or more factors not yet isolated in crystalline form. Factor 2,[206] factor W,[207] yeast filtrate factor,[208] or any one of several other preparations will apparently complete the growth requirements of the rat for the vitamin-B complex.

Considerable evidence has accumulated to indicate that pantothenic acid is a part of these various preparations. Hitchings and Subbarow[209, 210] have shown that a calcium salt preparation, which behaves like pantothenic acid in stimulating the growth of microörganisms, also markedly stimulates the growth of rats when fed at a level of 8 mg. per week to animals deficient in the filtrate factor. Macrae and coworkers[211] describe the preparation of a "pantothenic acid-like material" of which the rat requires 1.2 mg. per week. Hoffer and Reichstein[212] and El-Sadr and coworkers[213] report growth-stimulating effects of materials which show reactions similar to those of pantothenic acid. Black, Frost, and Elvehjem[214] report that their factor W preparations contain significant amounts of pantothenic acid and at least one additional growth factor rquired by the rat other than thiamin, riboflavin, and pyridoxine. Woolley[215] has shown that pantothenic acid promotes growth in a filtrate-factor-deficient animal for only a relatively short period of time. Young rats fed a basal ration plus thiamin, riboflavin, nicotinic acid, pyridoxine, β-alanine, and choline for 4 to 6 weeks no longer respond to additions of pantothenic acid, but "respond dramatically" to certain liver concentrates plus pantothenic acid.

Sure[165] reports that attempts to rear young of the albino rat on B-deficient diets supplemented with thiamin, riboflavin, pyridoxine, choline, and nicotinic acid result in complete failure about the tenth to fifteenth day of lactation. The further addition of the "W" factor of Elvehjem results in success in every case.

Evidence has been presented in a number of papers to indicate that the B complex may contain several other factors which play a role in normal nutrition. Among these are the following: anti-hemorrhagic adrenal necrosis factor,[216] B_w and B_x[217] or anti-grey hair factor,[218] two different lactation factors,[219] grass-juice factor,[220] B_4,[221] anti-spectacled eye factor,[191] anti-panmyelophthisis factor,[222] factor W,[214] factor 2,[206] year or liver filtrate factor,[211] vitamin H,[223] and probably several others. Inasmuch as these factors have not been isolated and identified, they are not included in this discussion.

CHOLINE*

Best and Huntsman[224] were the first investigators to demonstrate the action of choline in preventing the deposition of fat in the liver and in accelerating the rate of removal of fat from a "fatty liver."

McHenry[225] and Best, Mawson, McHenry, and Ridout[226] have shown that rats fail to gain in weight on diets low in choline (0.3 mg. per 100 gm. food) and suggest that choline may be an accessory food factor. They conclude that the minimum effective dose of choline is less than 3 mg. per rat per day. Maclean, Ridout, and Best[227] state that animals on a minimal amount of choline lose weight and store little fat in the usual fat deposits. When choline is given (33 to 35 mg. per rat per day) the weight gains and fat storage are normal.

Griffith and Wade[228, 229] have recently demonstrated that choline is a dietary essential without which young rats develop a severely toxic deficiency (within 10 days or less) characterized by "a marked hemorrhagic enlargement and degeneration of the kidneys, a regression of the thymus, and an enlargement of the spleen." The presence of extra cystine in the diet increases this effect while extra methionine delays its onset or prevents it entirely. These observations, together with those which concern the relation of choline, cystine, and methionine to the production of fatty livers,[230, 231] suggest a relationship between the metabolism of choline and that of the sulfur-containing amino acids. Further evidence of this relationship is found in the report by du Vigneaud, Chandler, Moyer, and Keppel[233] that when choline is fed with homocystine the rat can utilize this combination for growth purposes in lieu of methionine.

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* Choline is often discussed in connection with the vitamin B complex and is, therefore, included at this point.

Griffith[234] reports that "approximately 1 mg. of choline chloride is required per day to prevent renal lesions" and the requirement for the prevention of fatty livers is two to three times that amount. He suggests that there is a decrease in the choline requirement of rats over 30 days of age. Choline deficiency develops more slowly and is less severe in young female rats than in males of the same age and weight.

Sure[235] has demonstrated that choline is an indispensable component of the ration for growth and lactation of the albino rat. When choline is withheld from the mother's ration the nursing young develop "paralytic symptoms indistinguishable from B1 polyneuritis" and die at about the fifteenth to the eighteenth day of lactation. The early stages of this paralysis can be cured within one day by the administration of choline. The daily administration of 15 mg. of choline chloride to the mother is satisfactory for growth and lactation in most cases, but occasionally it is necessary to divide this dose between the mother and her young. In paired feeding experiments animals receiving 6 to 15 mg. choline chloride daily grow much better than controls consuming the same amount of food.

Apparently 1 mg. of choline chloride per day is sufficient to prevent renal lesions; 2 or 3 mg. per day will prevent fatty livers; and 15 mg. per day will permit satisfactory lactation.

VITAMIN D

Vitamin D cures or prevents rickets. However, its specific antirachitic activity is demonstrable, in the rat at least, only by feeding very unusual diets. In some experimental animals a deficiency of vitamin D alone, without great distortion of the diet, is sufficient to produce rickets, but with rats the calcium and phosphorus of the diet must also be restricted or fed in unusual proportions. Standard diets for the production of experimental rickets in the rat are vitamin-D-free and usually are low in phosphorus and possess a calcium to phosphorus ratio of about 4 or 5:1. In addition, it is necessary to safeguard the animals from exposure to sunlight, or even to unusual amounts of filtered daylight, since exposure to ultraviolet light will prevent or cure rickets.

Bethke, Kick, and Wilder[236] report an extensive study of the effect of vitamin D upon growth and calcification, using diets of widely varied calcium and phosphorus content. They conclude that the most favorable calcium to phosphorus ratio for growth and bone formation is between 2:1 and 1:1, at which point the vitamin-D requirements are at a minimum. Regardless of the concentration or ratio of calcium and phosphorus in the ration, the addition of vitamin D (irradiated ergosterol) increases the growth of the animals and the ash content of the bones. However, at ratios between 2:1 and 1:1 the growth and calcification are only slightly lower in the absence of vitamin D, but the greater the divergence from this ratio the greater the improvement produced by the addition of vitamin D (about 300 International Units per 100 gm. food).

Nicolaysen[237] has shown that the absorption of calcium from a diet nearly free from phosphorus is markedly reduced in vitamin-D-deficient rats, while the absorption of various forms of phosphorus from a diet nearly free from calcium is complete both in normal and in vitamin-D-deficient animals. The output of *endogenous* calcium in the feces is increased several times in vitamin D deficiency, but is not influenced by ingested phosphorus. The output of *endogenous* phosphorus is increased by the ingestion of calcium, but is increased more in vitamin D-deficient than in normal rats and is not affected by injections of calcium and phosphorus. The increased excretion of calcium in the feces in vitamin D deficiency, therefore, is the direct result of decreased absorption, while increased phosphorus excretion is the indirect result of its precipitation by the increased amount of calcium in the intestine. Vitamin D does not affect the absorption of calcium by altering the pH of the intestinal tract.[238]

Cohn and Greenberg[239] have shown that the absorption of phosphate by rachitic rats is increased but little by vitamin D, but the inorganic phosphorus uptake in the bone is increased 25 to 50%. They suggest that "vitamin D may act to aid the conversion of organic to inorganic phosphorus."

Nicolaysen and Jansen[240] report that the degree of calcification of bones, as indicated by chemical analysis, appears to depend solely on the amounts of calcium and phosphorus in the blood stream and not on the presence or absence of vitamin D in the diet (extra phosphate was introduced subcutaneously into rats receiving a low phosphorus diet). However, histological examinations showed that vitamin D may influence the architecture of the bones.

An increase in serum phosphatase has been suggested as the first definite evidence of the development of rickets, but similar increases appear in other diseases of the bone, indicating that this reaction may be a generalized response to a disturbed mineral metabolism rather than a specific response to a deficiency of vitamin D.[241, 241a]

Insofar as the teeth are a part of the bony structure of the body, their relation to vitamin D parallels that of other calcification processes (see Chapter 5).

Cox and Imboden[242] have reported an extensive study of reproduction on a diet of purified foodstuffs containing a constant quantity, but varying levels and ratios, of calcium and phosphorus. "No vitamin D was added and the animals were protected from ultraviolet light." Reproduction, growth, and calcification are excellent when adequate amounts and certain ratios of calcium and phosphorus are available, despite the very low amount or possibly complete absence of vitamin D in the ration.

Shohl[243] in his review, concludes that in all cases in which the amounts of calcium and phosphorus are sufficient and the disproportion not too great normal bones are produced and extra vitamin D is not needed by the rat.

There is very good evidence that the rat can survive satisfactorily in the complete absence of dietary vitamin D provided the amounts of available calcium and phosphorus of the ration are within certain limits. When small amounts of vitamin D (3 U.S.P. units per gram of food) are added to such a diet, growth and calcification may be more rapid, but the difference is usually quite small. However, in the presence of vitamin D a much greater range of variability in the content of calcium and phosphorus in the diet is possible without any deleterious effect upon the development of the animal. The addition of vitamin D makes a more efficient utilization of dietary calcium and phosphorus possible, especially when their dietary supply is inadequate or disproportionate. Within rather wide limits the greater the divergence of dietary calcium and phosphorus from the optimum the greater the requirement for vitamin D in order to permit normal calcification and growth.

VITAMIN E

In 1922 Evans and Bishop[244] discovered a dietary factor, subsequently called "vitamin E," which is particularly concerned with reproduction in the rat. A deficiency of vitamin E in the female rat does not interfere with estrus, ovulation, or impregnation of the ovum, but this deficiency does cause death and resorption of the fetus before maturity is reached. If a female rat on a vitamin-E-deficient diet is given a single dose of vitamin E a few days after mating, or given sufficient vitamin E during the gestation period, normal young are produced. A deficiency of the vitamin in the male rat causes degeneration of the germinal epithelium which results in sterility. This damage appears to be irreparable.[245]

Emerson and Evans[246, 247] report that vitamin-E-low female rats require increasing amounts of vitamin E with increasing age in order to restore fertility. From 3 to 6 months of age, a single dose of 0.5 Gm. of wheat germ oil to females invariably leads to the birth of normal-sized litters of living young. After the seventh month only about half the animals give birth to living young with this dosage (0.5 Gm.), and these are undersized litters. By the eleventh month practically no animals respond to 0.5 Gm., but almost a third do respond to 4.0 Gm., producing small litters of living young. After the fifteenth month of life no response can be secured with a twentyfold increase of dosage.

Blumberg,[248] Martin,[249] Ringsted,[250] Emerson and Evans,[251] Olcott and Mattill,[252] and others have demonstrated that a vitamin-E-deficient diet produces a decrease in the rate of growth of the rat. Considerable variation exists in the reported time of onset and degree of effect of this deficiency upon growth, but all investigators agree that the addition of wheat germ oil to the diet permits an improvement in growth.

Evans, Emerson, and Emerson[253] report that groups of male rats receiving an E-free diet supplemented with 0.75 mg. of alpha-tocopherol daily show normal sexual behavior when mated with normal females, and grow as well as animals on the stock diet. Those without the supplement show a decrease in growth rate after the fourth month and fail to sire litters when placed with normal females. Female rats raised from weaning on an E-free ration plateau in weight at about four months of age. Growth is resumed when 1 mg. of alpha-tocopherol is administered daily.[254] A daily dose of 1 to 3 mg. permits females of proved sterility to bear living young. Either 500 mg. of wheat germ oil or 3 mg. of alpha-tocopherol will enable young mature female rats which have been reared and held on an E-free diet to give birth to normal-sized litters of living young.[255]

Evans and Burr[256] were the first investigators to demonstrate paralytic symptoms in suckling young of female rats which had received only enough vitamin E to produce a litter. This condition is characterized by severe degeneration of the skeletal muscles.[257, 258, 259] Telford, Emerson, and Evans[257] report, also, that a marked loss in skeletal-muscle creatine occurs during this paralysis. Goettsch and Ritzmann[259] have demonstrated that a total of 5 mg. of alpha-tocopherol given to the young during 15 days will protect them from this disorder, while 0.5 mg. gives only partial protection. Evans and Emerson[260] report that this muscular dystrophy of the young can be prevented by the administration of 10 mg. of alpha-tocopherol to the mother on the day of littering or by the feeding to the young of 1 mg. daily, from the 10th day, or 3 mg. daily from the 15th day. The administration of 3 mg. daily from the 18th day is ineffective.

Animals reared and maintained upon E-low rations also develop a characteristic disability in locomotion, or partial paralysis.[250, 261] Evans, Emerson, and Telford[262] report that animals maintained for 22 months on a diet devoid of vitamin E weigh almost 100 Gm. less than their controls and show severe muscular dystrophy. Mackenzie, Mackenzie, and McCollum[263] state that rats reared and maintained on a highly purified vitamin-E-deficient diet develop paralysis of the hind legs accompanied by tremors and incoördination of the forelegs and head. Knowlton, Hines, and Brinkhous[264] have shown that subcutaneous injections of 3 mg. of alpha-tocopherol acetate per week are effective in both the cure and the prevention of the nutritional muscular dystrophy seen in rats maintained from an early age on a vitamin-E-deficient diet.

About 1 mg. of alpha-tocopherol per day is sufficient for normal growth of the rat; 3 mg. per day permits gestation; and a single dose of 10 mg. to females on the day of littering will prevent paralysis of the suckling young.

VITAMIN K

Dam and Schønheyder[265] and Almquist and Stokstad[266] and others have described a dietary deficiency disease of birds characterized by a prolonged blood clotting time and a marked tendency to hemorrhage (subcutaneous, intramuscular, and abdominal). In general, attempts to produce the disease in rats have been unsuccessful.

Greaves[267] reports that only 12 of 77 animals reared and maintained on a vitamin-K-free diet developed hemorrhagic tendencies and exhibited prothrombin values below 50% of normal. These animals promptly returned to normal when treated with a vitamin-K concentrate. The remaining 65 animals used in these studies grew, reproduced and raised young in a normal manner. Greaves[268] has also shown that bile-fistula or jaundiced animals exhibit marked bleeding tendencies associated with low blood prothrombin values, which condition can be cured by the administration of vitamin-K concentrates. Bile salts are essential to the intestinal absorption of vitamin K in the rat.

According to Dam and Glavind[269] 7 out of 15 rats receiving a K-deficient diet developed a severe K-avitaminosis. The authors suggest that an alteration in the bacterial flora in the intestine must take place before the animals develop symptoms of this deficiency.

SUMMARY OF KNOWN REQUIREMENTS

Calcium and Phosphorus. The requirements for optimum growth, reproduction, and calcification are supplied by a diet containing about 0.5 to 0.6% calcium and the same or a slightly lower concentration of phosphorus. This corresponds to an average daily intake of about 40 to 50 mg. of calcium and 35 to 45 mg. of phosphorus.

Potassium. Males require a minimum of 15.0 mg. and females 8.0 mg. of potassium per day for normal growth. Diets containing 0.14% potassium are adequate for pregnancy and 0.5% for lactation.

Sodium. Diets containing about 0.5% sodium appear to be the most satisfactory for growth and reproduction.

Chlorine. About 5 mg. of chlorine per day are sufficient for growth, gestation, and lactation.

Iron. About 0.25 mg. of iron per day is sufficient for hemoglobin synthesis; 5 mg. per day are probably more than adequate for reproduction and lactation.

Copper. It appears that 0.05 mg. of copper is about the minimum daily requirement for growth and reproduction, but probably 0.10 mg. is more nearly the optimum intake.

Iodine. Diets supplying about 1 to 2 micrograms of iodine per day are minimal, and those containing about 265 micrograms per kilogram are quite adequate to protect the growing rat from iodine deficiency.

Magnesium. At least 4 mg. of magnesium are required per kilo of body weight per day, or a little more than 5 mg.% of the diet, for normal development and reproduction.

Manganese. About 0.5 mg. of manganese per day is minimal, while 0.8 mg. is adequate for excellent reproduction. Good lactation is possible on a diet of milk containing only 0.03 mg. of manganese per liter.

Zinc. Normal growth is possible on diets supplying 40 micrograms of zinc per day.

Cobalt. If cobalt is required, 0.4 microgram per day is sufficient.

Aluminum. If aluminum is required, 1 microgram per day is sufficient.

Arsenic. If arsenic is required, 2 micrograms per day are sufficient.

Boron. If boron is required, 0.8 microgram per day is sufficient.

Bromine. If bromine is required, 5 parts in 10 million of diet support growth.

Fluorine. If fluorine is required, 1 part in 10 million of diet is sufficient.

Protein. Optimum growth and excellent reproduction and lactation occur on diets containing 25 to 30% of protein, with possibly even better reproduction on a ration containing 40% protein.

Amino Acids. (See table in section on Amino Acids and Proteins.)

Fatty Acids. Growth and gestation appear to be normal in animals receiving 25 mg. of methyl linoleate per day, but lactation is unsatisfactory unless significant amounts of fat are also present in the diet.

Vitamin A. About 4 micrograms of A or 15 to 20 micrograms of carotene per kilo of body weight per day are sufficient to prevent vaginal cornification. About 40 micrograms of carotene 3 times per week permit reproduction.

Thiamin. Apparently 1 microgram of thiamin per gram of food or 10 micrograms per day are sufficient for growth, but 120 micrograms are required per day for reproduction and lactation.

Riboflavin. Although 40 micrograms of riboflavin per day are sufficient for optimum growth, 120 micrograms are required for satisfactory rearing of young.

Nicotinic Acid. Apparently nicotinic acid is not required by the rat.

Pyridoxine (B_6). About 10 micrograms of pyridoxine per day are sufficient for normal growth, but at least 50 micrograms are required for satisfactory lactation.

Other Factors. Until these factors are identified, their requirements cannot be determined.

Choline. Apparently 1 mg. of choline chloride per day prevents renal lesions; 2 or 3 mg. per day prevent fatty livers; and about 15 mg. per day permit satisfactory lactation.

Vitamin D. Apparently vitamin D is not required if the Ca:P ratio is between 1:1 and 2:1 and about 0.5% phosphorus is present in the diet, while diets outside this range are improved by the addition of 3 U.S.P. units per gram of food.

Vitamin E. About 1 mg. of alpha-tocopherol per day is sufficient for normal growth; 3 mg. per day permit gestation; and a single dose of 10 mg. to females on the day of littering will prevent paralysis of the suckling young.

Vitamin K. Nearly all rats grow and reproduce normally in the absence of this vitamin.

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6

The Teeth

By

ISAAC SCHOUR, D.D.S., Ph.D., and MAURY MASSLER, D.D.S., M.S.

INTRODUCTION

The tooth is more than a masticatory organ. Because of its peculiar properties it is a valuable biologic indicator which mirrors and records, during its development, the metabolic status of the animal. This record, unlike similar records in the bone, is not transitory but is permanently engraved upon the enamel and dentin in which resorption does not occur. Studies of the experimental effects upon the teeth therefore throw light on the general physiology of the animal as well as on various phases of dental physiology, and are of more than didactic interest to the experimenter.

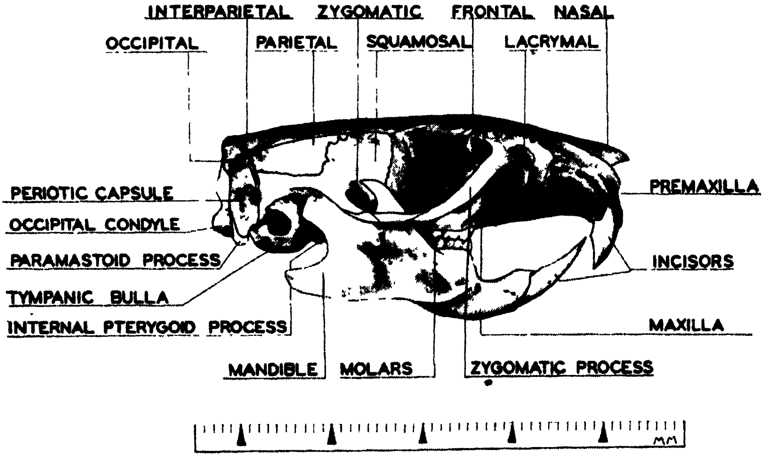

Fig. 55. Right lateral aspect of the dissected skull of a white rat 300 days of age (× 1½). (Spence.[29])

The dentition of the rat is monophyodont (one set of teeth). It consists of

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one incisor and three molars in each quadrant of the jaws and combines two types of teeth in one animal. The incisor is situated anterior to the

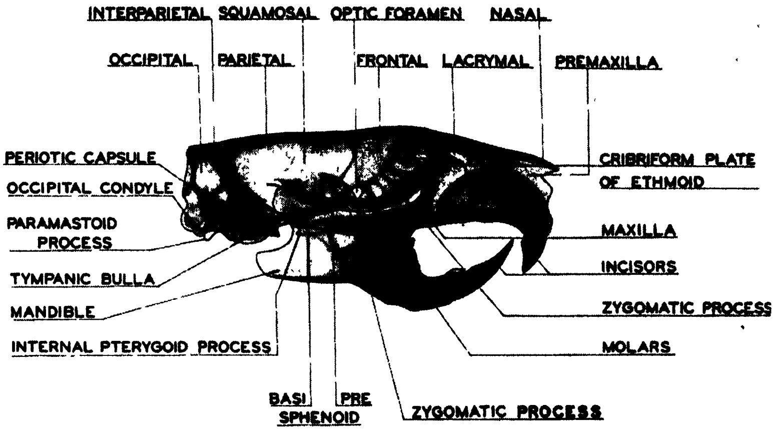

Fig. 56. Roentgenogram of the right lateral aspect of the same rat as in Fig. 55. Note the difference in the relation of the molar teeth to the upper and lower incisors. The enamel and dentin of the incisors and the bony outlines are traced in black (X 1½). (Spence.[29])

molars in the upper jaw and extends below and beyond the molars in the lower jaw (Figs. 55 and 56).*

The incisors grow, calcify and erupt continuously throughout the life of the animal and therefore show in one tooth the complete life cycle of tooth development from inception to maturity (Figs. 63 and 64). They are also continuously worn away by attrition at their incisal edges. In the adult rat the records engraved upon the enamel and dentin of the incisor are therefore limited to the 40 or 50 days preceding.

The molars resemble the more highly developed human tooth type and are limited in development chiefly to the first 125 days of life. They are abraded very slowly so that the records engraved upon them during their development may be read long afterwards in the adult. We have thus two types of kymo-

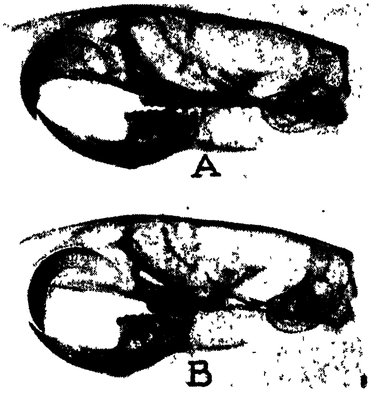

Fig. 57. Roentgenograms of the skull of a rat showing (A) lower incisor in posterior relation to upper and (B) lower in anterior relation to upper (natural size).

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* The description of the rat incisor applies for the most part to the incisors of all rodents. In rodents of mixed diet such as the common rat or the mouse the molars are of limited growth and have distinct roots. They resemble somewhat minute human molars. In those rodents whose food is herbivorous in character (guinea-pig and rabbit) the molars grow from persistent pulps.

graphic readings. In the incisor the continuously revolving record is destroyed in 40 to 50 days by attrition, whereas in the molar the record, though not continuous, is more nearly permanent and is available for reference at any later date (Table VI).

TABLE VI

Chronology of the development of the dentition of the albino rat[1]

Developmental Process	Incisors	Molars[2]		
		M-1	M-2	M-3
Initiation (1st Appearance of Dental Lamina)	14th day in utero	13th day in utero	14–15th day in utero	20th day in utero
Histo-differentiation of Ameloblasts Begins	18–19th day in utero	20th day in utero	21–22nd day in utero	8–10th day
Apposition of Dentin Begins[3]	20–21st day in utero	20–21st day in utero	1–2nd day	13–14th day
Crown Completed (Amelogenesis Ends)	Growth continuous	11th day	13th day	21st day
Bifurcation of Roots Begins	None present	14–16th day	16–18th day	24th day
First Appearance in Oral Cavity	About 10th day	19th day	22nd day	35th day
Functional Occlusion	16th day	25th day	28th day	40th day
Beginning of Pigmentation	28–30th day	———NO PIGMENTATION———		

[1] Data abstracted from: Addison and Appleton[2], Glasstone[3], Mellanby[4], Hoffman and Schour[5]

[2] Upper teeth are chronologically about 24 hours behind the lower.

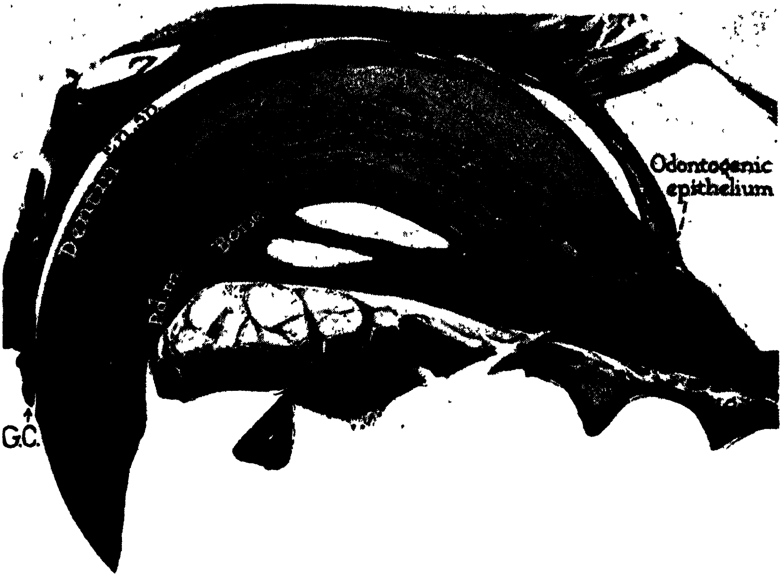
[3] Amelogenesis begins 12–24 hours later.

HISTOGENESIS AND HISTOPHYSIOLOGY OF THE TEETH

Rat Incisor

Morphogenesis. The form of the rat incisor is that of a segment of a laterally-sheared logarithmic spiral. The curvature of the tooth is smooth and represents in the upper incisor a greater segment of a smaller spiral (about 210°), and in the lower, a smaller segment of a larger spiral (140-145°) (Figs. 58 and 59). The upper and lower incisors are thus different segments of the same logarithmic spiral (Fig. 66).

The incisor has no root but consists of a powerful crown which may be divided longitudinally into two parts, the labial or convex portion covered with enamel and the remaining lingual or concave and lateral portions covered with cementum (Figs. 58, 59, and 60). The curved dentin cone which constitutes the bulk of the tooth is compressed medio-laterally (Fig. 60) and has a slight lateral shear (Fig. 70). During function the apex of the cone is worn away by attrition (functional wear). The alternate backward and forward movement of the jaws in gnawing produces a much more rapid wearing of the relatively softer cementum and dentin on the lingual side than of the very



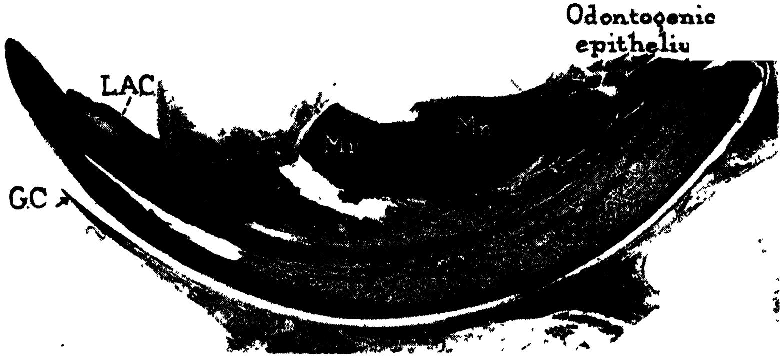


Fig. 58. (*Top*) Photomicrograph of a midsagittal section of an upper incisor of a normal rat 65 days of age (× 7). Al. b., alveolar bone; En. ep., enamel epithelium; En. sp., space formerly occupied by enamel lost in decalcification; P.D.M., periodontal membrane; G.C., gingival crest. Note the calcific mass at the extension of the pulp to incisal edge. Note too the greater labio-lingual width in the proximal than in the distal portion, a result of the conical form of the tooth. (From Schour and Steadman.[7])

Fig. 59. (*Bottom*) Photomicrograph of a longitudinal section of a lower incisor of a normal rat about 60 days of age (× 5). The section fails to pass through the center of the tooth near the incisal edge because of the lateral shear of the tooth in three planes of space. Note the difference in curvature in the upper and lower incisors. G.C., gingival crest; L.A.C., labial alveolar crest; Mr., molar.

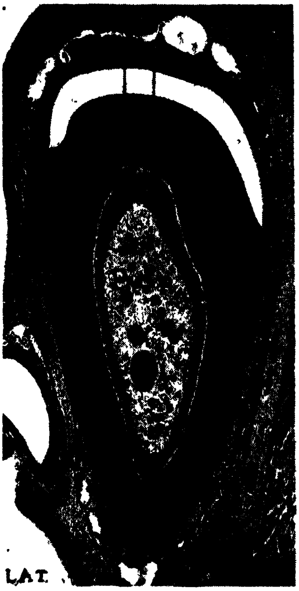


Fig. 60.

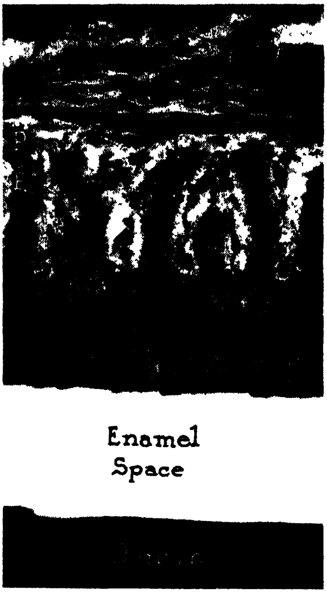


Fig. 61.

Fig. 60. (*Left*) Photomicrograph of a cross-section of an upper incisor of a rat 65 days of age (× 23). The section was cut at the level of the middle third of the tooth. Alv. B., alveolar bone; Od., odontoblasts; P.D.M., periodontal membrane. Compare with Fig. 58. The dentin is not homogenous in calcification but interglobular and marbled. The basal diet which was considered to be normal had an insufficient amount of Vitamin D. White area over the dentin is the space occupied by the enamel before it was lost by decalcification.

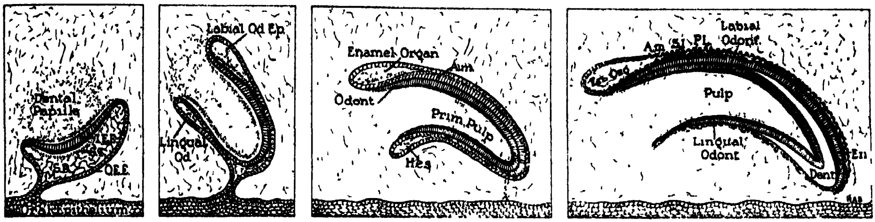
Cementum Cementum is present on the lingual and that part of the lateral surface not covered with enamel. It is very thin (about 3–4 μ). The fibers of the periodontal membrane become embedded in the growing cementum and thus attach the tooth to the surrounding alveolar bone.

Periodontal Membrane. The periodontal membrane is composed of three layers of principal fibers: (a) the cementum-embedded fibers, (b) the bone-embedded fibers, and (c) the plexus intermedium. This arrangement probably allows for the continuous eruption of the tooth and at the same time holds the tooth firmly within the alveolar socket. It also contains fibroblasts, cementoblasts, osteoblasts, interstitial fibers, nerve fibers and wandering cells. The blood supply is rich and lies closer to the alveolar bone. The vascularity of periodontal membrane, particularly of the basal portion, is noteworthy inasmuch as it serves as a pneumatic trough which absorbs the piston-like shocks of incisal stress (Boyle, 1938).

Labial Alveolar Periosteum This periosteum differs from the periodontal membrane in that it covers enamel instead of cementum. Its fibers are arranged parallel with the long axis of the tooth.

Fig. 61. (*Right*) Higher magnification of corresponding region indicated by square in figure 60 of normal rat 250 days of age (× 267). Am., ameloblasts; L.A.P., labial alveolar periosteum; Pap., papillary layer. (From Schour in Cowdry's Special Cytology, 1932.)

The ameloblasts maintain their columnar shape to about the middle third of the tooth where they become gradually reduced and finally atrophy at about 50 days of age. In the very young rat, because of the small size and faster rate of eruption of the tooth, the ameloblasts maintain their columnar shape even up to the gingival crest. The enamel (see Fig. 78) consists of two layers, a thin outer fibrous and a thicker inner plexiform layer, about 30 and 70 microns respectively in the upper and 20 and 120 microns in the lower incisors of a 5-month rat (Addison and Appleton, 1915).



17 days in utero
A

19 days in utero
B

20 days in utero
C

2 days postnatal
D

Fig. 62. Schematic representation of the growth of the upper incisor of the rat.

(A) Bud Stage in the proliferative growth of the enamel organ from the oral epithe'ium The enamel organ consists of the inner enamel epithelium (I E.E), stratum intermedium (S I), stellate reticulum (S R) and outer enamel epithelium (O E E.).

(B) Cap Stage and (C) Bell Stage Note the differentiation of the formative cells and the outline of the size and shape of the future tooth. Od. Ep., odontogenic epithelium, Am , ameloblasts, H E S , anlage of Hertwig's epithelial sheath; Odont., odontoblasts, Prim. Pulp, primitive pulp

(D) Beginning of matrix deposition. Enamel (En) black, dentin (Dent) white Note the bulbous portion of the odontogenic epithelium on the labial, the true enamel organ (En org) The bulbous portion of the odontogenic epithelium on the lingual is the anlage of Hertwig's Epithelial Sheath (H E S) Note the greater labial than lingual length and curvature of the tooth. (Adapted from Addison and Appleton [2]) Am , Ameloblasts, Odont , Odontoblasts, P L., Papillary Layer, S I., Stratum Intermedium, S R , Stellate Reticulum

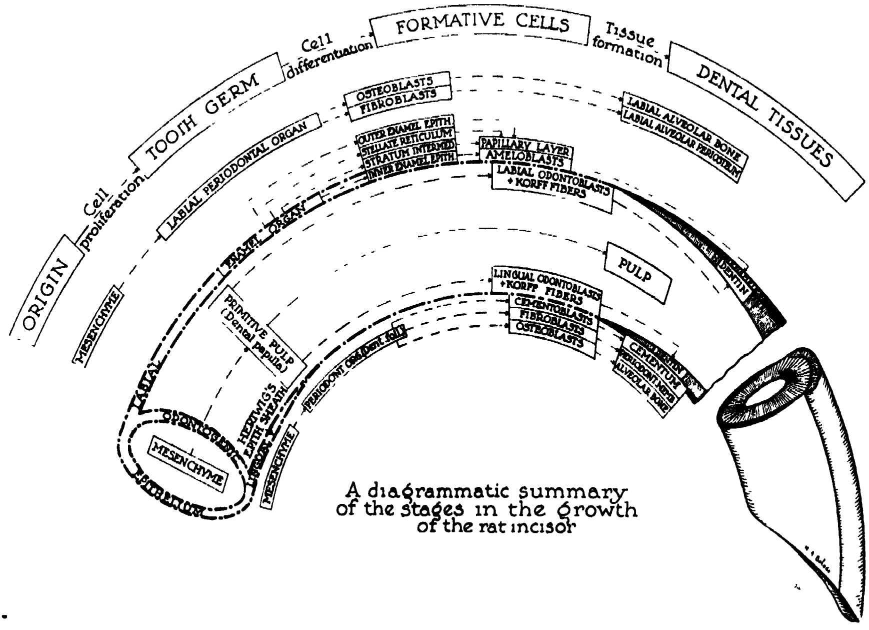


Fig. 63. A diagrammatic summary of the stages in tooth development indicating the origin and relationships between the formative cells and the dental tissues.

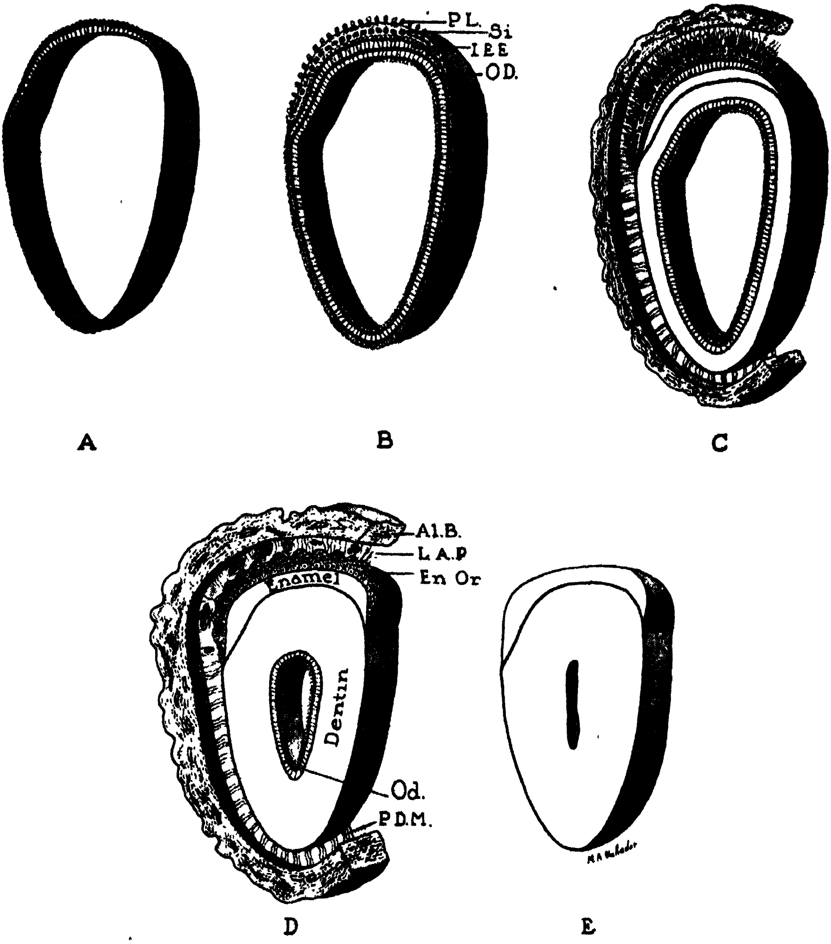


Fig. 64. Diagrammatic representation of tooth development as seen by successive cross-sections through an upper rat incisor. Compare with Fig. 63.

(A) Cross section through the basal end showing ring-like arrangement of the inner layer of the odontogenic epithelium Note that the shape of the incisor is outlined The cells on the labial are tall columnar and will differentiate into ameloblasts

(B) Cross section immediately anterior to A, prior to appositional growth. Note the differentiation of the enamel organ which now consists of tall inner enamel epithelium (I E E), a stratum intermedium (Si), and a papillary layer (P.L) On the inner circumference of the epithelial ring the peripheral layer of mesenchymal cells of the pulp have differentiated into the tall columnar odontoblasts (O D)

(C) and (D) Successive cross sections anterior to B, showing enamel and dentin formation The alveolar bone (Al B) encircles the tooth, and is removed from the lateral surface. Note that the labial aspect of the tooth is covered with enamel, the remainder with cementum. The periodontal membrane fibers (P D M.) are embedded in alveolar bone and cementum. The labial alveolar periosteum (L A P) fills the area between the alveolar bone and the papillary layer of the enamel organ (En Or) In (D), enamel formation is completed and the enamel organ and ameloblasts are atrophied Od , odontoblasts.

(E) Cross section of the intra-oral portion of the tooth near the incisal edge Note the pulpal slit

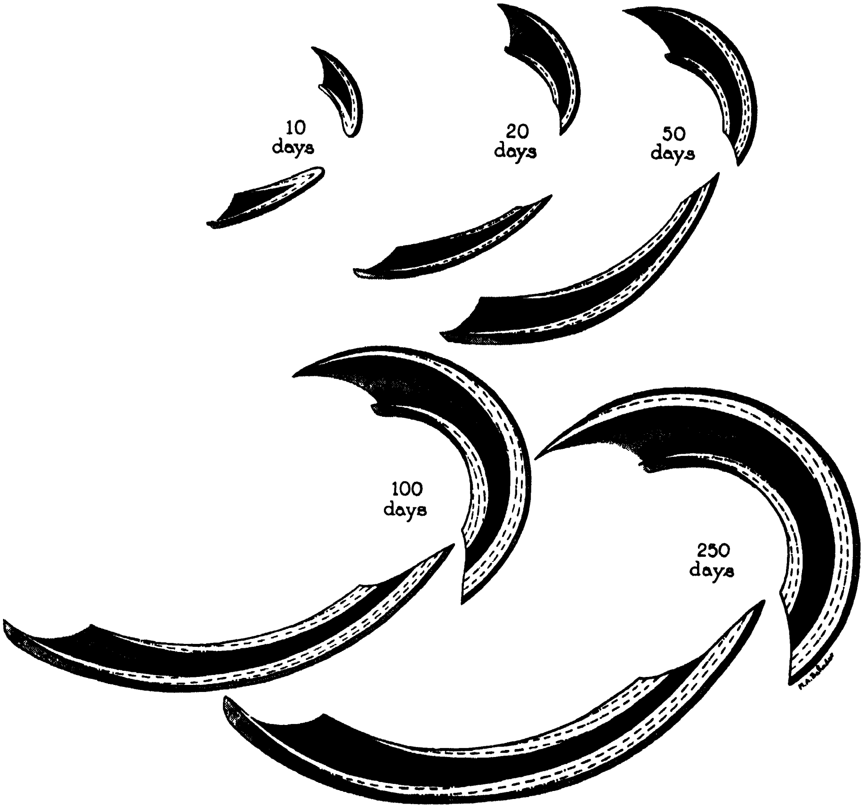


Fig. 65. Diagrammatic representation of the development of the rat incisors as seen by longitudinal hemisections of the teeth at successive ages. Note the conical form and increased size of the teeth as the animal becomes older. The incremental pattern of the dentin is indicated by dotted lines. (From Herzberg and Schour.[1])

hard enamel on the labial surface, and results in a sharp chisel-shaped edge (*Fig.* 57).

The tooth develops from an elliptical sheath called the odontogenic epithelium which is located at its base and which encloses the connective tissue of the primitive pulp (Figs. 62-64). This epithelial ring determines the cross-sectional size and outline of the future tooth and proliferates throughout the life of the animal. Up to at least 150 days of age it increases in size at a constant ratio with the age of the animal so that the diameter of the tooth at any given level is an index to the age of the animal.[1] (Figs. 65-67.)

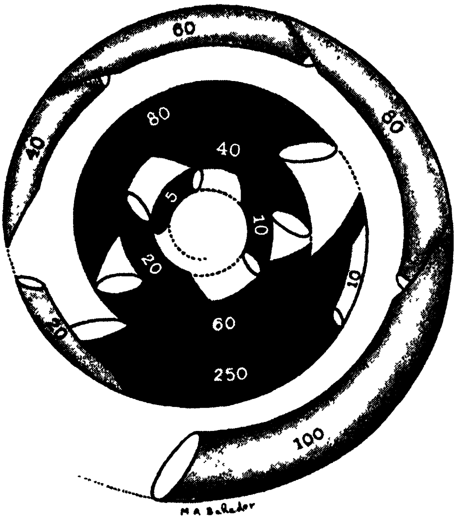


Fig. 66. Successive segments of upper (dark) and lower (light) incisors of rats of different ages, arranged in a continuous spiral form. Figures indicate days of age. This form would be obtained in the absence of attrition ("elongation"). Note that the upper and lower incisors are different segments of the same logarithmic spiral. (From Herzberg and Schour, 1941.)

Life Cycle. The tooth undergoes four distinct stages and processes of development:

1. Growth
   a. Initiation and proliferation of the enamel organ from the oral epithelium,
   b. Morphodifferentiation and histo-differentiation of the formative cells,
   c. Apposition or extra-cellular deposition of the enamel, dentin, cementum and bone matrices.
2. Calcification of the deposited matrix
3. Eruption of the formed and calcified tooth into the oral cavity
4. Functional attrition

This classification places emphasis on the physiologic and dynamic aspects of development rather than the morphologic. Thus gross, radiographic or

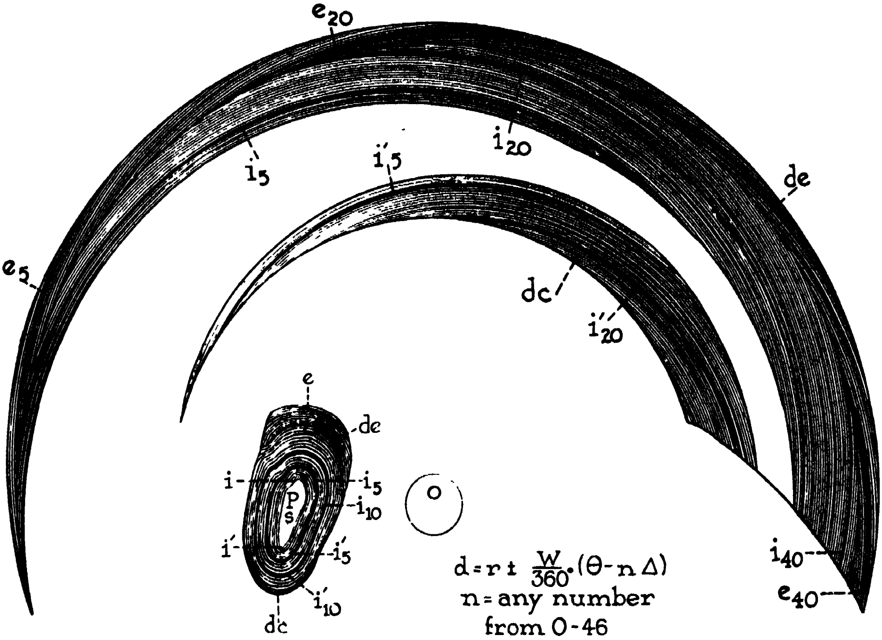


Fig. 67. Geometric construction of the incremental pattern of the enamel and dentin of the upper incisor of the rat using a single center O according to the formula indicated. The gradient of growth is very small so that an archimedian rather than a true logarithmic spiral is shown. d.c., dentino-cemental junction; d.e., dentino-enamel junction; e., incremental lines in enamel; i., incremental lines in labial dentin; i'., incremental lines in lingual dentin. The numbers indicate the time of formation in number of days before death. Insert shows cross sectional ring pattern through the middle of the incisor. Ps., pulpal space. (From Schour and Steadman.[7])

The odontoblasts migrate toward the distal ends of the tooth and at the same time recede pulpally so that the dentin increases in width anteriorly with a corresponding reduction of the enclosed richly vascular pulp. The pulpal recession of a given odontoblast is in direct proportion to the amount of dentin laid down Experimental studies have shown that dentin is laid down at the rate of 16 μ in twenty-four hours (Schour and Steadman, 1935) The forward movement of the odontoblast is in direct proportion to the rate of eruption (about 2 mm per week in the upper incisor) Thus an odontoblast five days old has receded approximately 80 microns (5x16 μ) centrally to position i 5 and about 1500 μ anteriorly. Succeeding odontoblasts align themselves next to their predecessors and aid in the deposition of new dentin matrix The result is the regular and ryhthmic daily deposition of layers of dentin 16 μ in thickness in the form of truncated cones, one within the other, at the expense of the enclosed pulp

As the tooth erupts the dentin layers move anteriorly with the tooth and are worn off but are continuously replaced by similar layers behind them so that the tooth rolls forward like the drum of a kymograph

The cementum-covered dentin begins its apposition at a more incisal level than does the enamel-covered dentin (Figs 58, 59, and 62) The rate of apposition is also slightly less on the lingual than on the labial, although this difference is very small (Herzberg[1])

The primary life-span of the dentin-forming cells is limited (about 40 days) so that the primary dentin is limited in width and never completely obliterates the pulp except in pathologic conditions (Fig 98) The incisal third of the dentin is therefore parallel to the dentino-enamel and dentino-cemental junctions The pulp can be seen as a slit at the incisal edge (Fig 63). However, the pulpal tissue is not exposed since a mass of calcific material fills in and replaces the vital pulp at the incisal edge (Fig. 58) histologic observations are not ends in themselves but are significant only inasmuch as they throw light on physiologic, or pathologic processes of growth, calcification, eruption, and attrition.

Growth. The various stages in the growth of the rat incisor are diagrammed in Figures 62 to 67. A more detailed description of the stages of proliferation, histo-differentiation, and appositional growth is given by Erdheim,[6] Addison and Appleton,[2] Schour and Steadman,[7] Noyes, Schour and Noyes,[8] Schour,[9] and Mellanby.[10]

Calcification. The calcification of the tissue matrix follows its deposition in close succession. The mechanism of the calcification process is but little understood. It is probably a physico-chemical precipitation of calcium salts in a protein medium (the tissue matrix) with the resultant calcospherite formations and Liesegang phenomenon (Figs. 68 and 69). For a discussion of the calcification process in teeth the reader is referred to Noyes, Schour and Noyes,[8] Schour,[9] Schour and Hoffman,[11, 12] Widdowson,[13] and Diamond and Weinmann.[14]

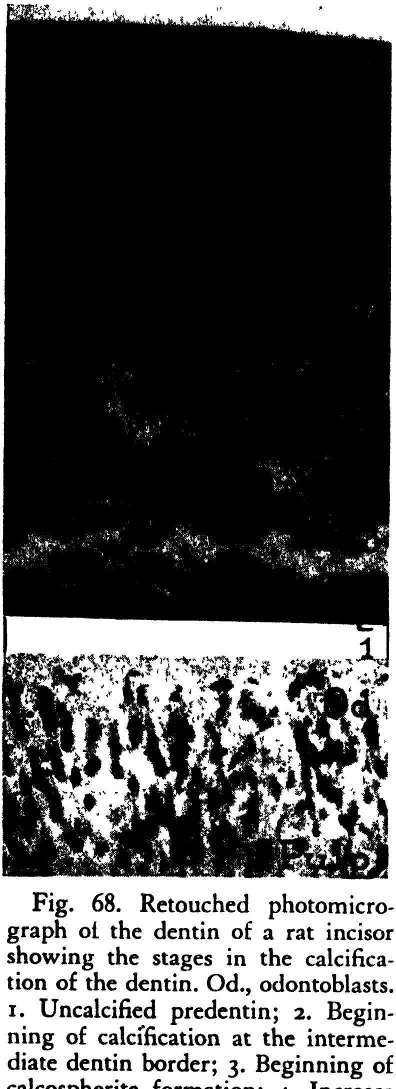


Fig. 68. Retouched photomicrograph of the dentin of a rat incisor showing the stages in the calcification of the dentin. Od., odontoblasts. 1. Uncalcified predentin; 2. Beginning of calcification at the intermediate dentin border; 3. Beginning of calcospherite formation; 4. Increase by concentric lamination; 5. Approximation and beginning of fusion of individual calcospherites. Stages 4 and 5 illustrate interglobular dentin; 6. Incomplete fusion of calcospherites (Marbled Dentin); 7. Fusion of calcospherites into a homogenous stratum; 8. Rhythmic alternation of stratification; 9. Mantle Dentin, the very well calcified layer at the dentin surface.

16 μ Calcification Rhythm. The calcification of enamel and dentin is rarely homogenous. Daily rhythmic alternations of dense and less dense calcification result in daily incremental ring formations 16 μ apart which facilitate the dating and measuring of experimental zones.[11] (Fig. 79.)

As the layers of enamel and dentin are formed and calcified they move forward and record the rate of growth and register changes in calcium metabolism just as the rings in the tree indicate seasonal growth, alternate periods of rainfall and drought, and the vicissitudes in the life history of the tooth.

Since no calcium withdrawal is possible in these tissues, these effects are recorded permanently, the enamel and

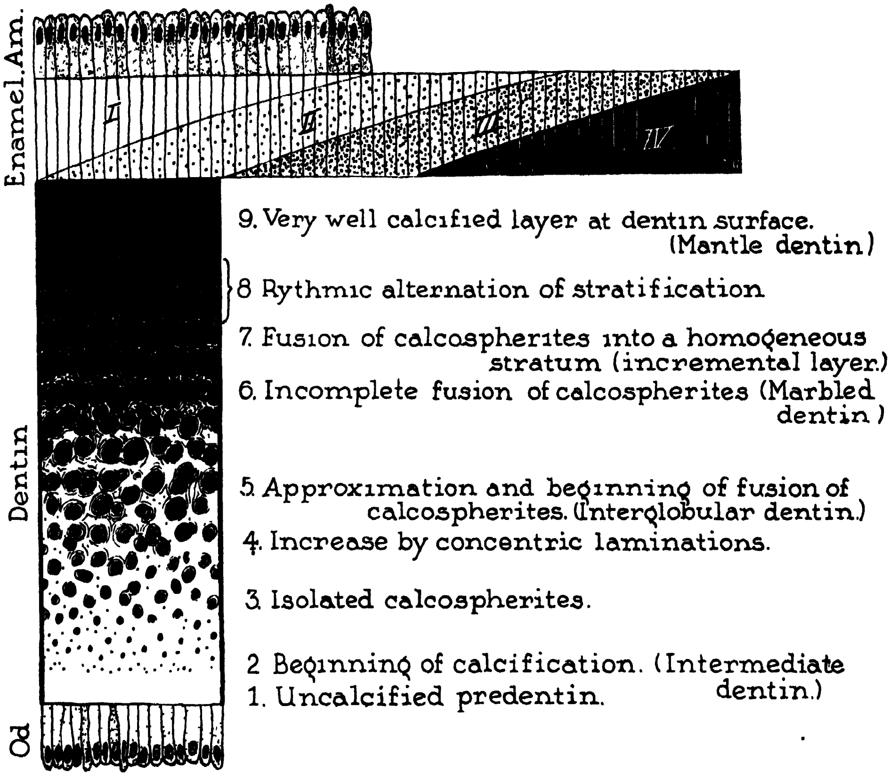

Enamel. Am.
I
II
III
IV
9. Very well calcified layer at dentin surface. (Mantle dentin)
8 Rythmic alternation of stratification
7. Fusion of calcospherites into a homogeneous stratum (incremental layer)
6. Incomplete fusion of calcospherites (Marbled dentin)
5. Approximation and beginning of fusion of calcospherites. (Interglobular dentin)
4. Increase by concentric laminations.
3. Isolated calcospherites.
2 Beginning of calcification. (Intermediate dentin)
1. Uncalcified predentin.
Dentin
Od

Fig. 69. A diagrammatic representation of the calcification of dentin and enamel. Am., ameloblasts; Od., odontoblasts.

I. Primary enamel—uncalcified acid resistant matrix. Stains blue with Mallory's connective tissue stain.

II. Young enamel—partly calcified but still acid resistant matrix. Stains orange with Mallory's connective tissue stain.

III. Transitional enamel—more calcified but for the most part still acid resistant. Stains blue with Mallory's connective tissue stain.

IV. Mature enamel—fully calcified and acid soluble. Lost during decalcification.

In the calcification of the enamel, the individual calcospherites form within the ameloblasts and fuse, and are deposited as the enamel globules. The matrix then calcifies in the stages shown above. These stages can be discerned by staining with Mallory's triple connective tissue stain or with Hematoxylin and Eosin.

Compare the steps of the calcification of the dentin (1-9) with Fig. 68.

TABLE VII

Maximal thickness of enamel and dentin of upper and lower rat incisors at different ages. Average of measurements taken in 60 animals at about the level of the alveolar crest. *(Courtesy of Dr. F. Herzberg)*

Age in Days	Enamel Thickness (in μ)		Dentin Thickness (in μ)			
	Upper	Lower	Upper		Lower	
			Labial	Lingual	Labial	Lingual
10	45	80	150	110	180	160
30	75	105	315	280	340	280
60	80	120	570	530	435	300
100	90	130	775	715	475	405
150	100	155	1150	775	695	570
200	100	155				

dentin acting like the drum of a kymograph which is fixed as soon as the stylus has written.

Eruption. Eruption is the process whereby the tooth is extruded into the oral cavity by a force which apparently acts at its basal or formative end and is probably related to the vascularity and the tissue tension located there.

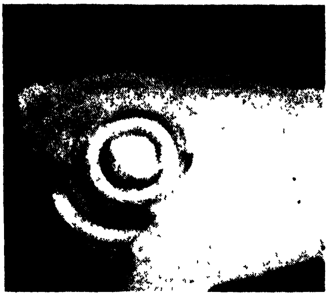


Fig. 70. Spiral elongation of rat incisors due to the failure of attrition. (From Schour.[9])

Prior to the actual eruption of the incisor the basal end grows backward into the jawbone which resorbs before its advance (Fig. 62). At 8 days of age actual eruption begins in a forward direction[2] and the incisal tip appears in the oral cavity at about the 8-10th day. The rate of eruption is about 2.1 mm. in the upper and 2.8 mm. in the lower incisors.[2, 15]

The rate of eruption of the tooth can be affected by local as well as systemic factors. When a tooth is fractured so as to remove the incisal stresses which normally oppose the eruptive forces, its eruption is increased up to two and three times the normal rate and the tooth elongates.[16, 107] (Fig. 70.) In hypophysectomy, the retardation and final cessation of eruption may be correlated with the concomitant marked reduction in the vascularity of the surrounding tissues.[17, 18]

Functional Attrition. Attrition may be defined as the normal wearing of the teeth due to functional activity. During function the incisal tips of the incisors are worn away. Since the enamel is harder than the dentin and cementum, the latter are worn away at a faster rate and a sharp bevel is produced which is a replica of the bevel of a hypodermic needle. (Fig. 65.)

When by reason of accident one of the incisors is broken or when a malocclusion occurs the non-attrition of the opposing incisor and its continuous growth results in an elongation or so-called overgrowth. The term "overgrowth" is misleading. In reality this elongation is not related to the rate of

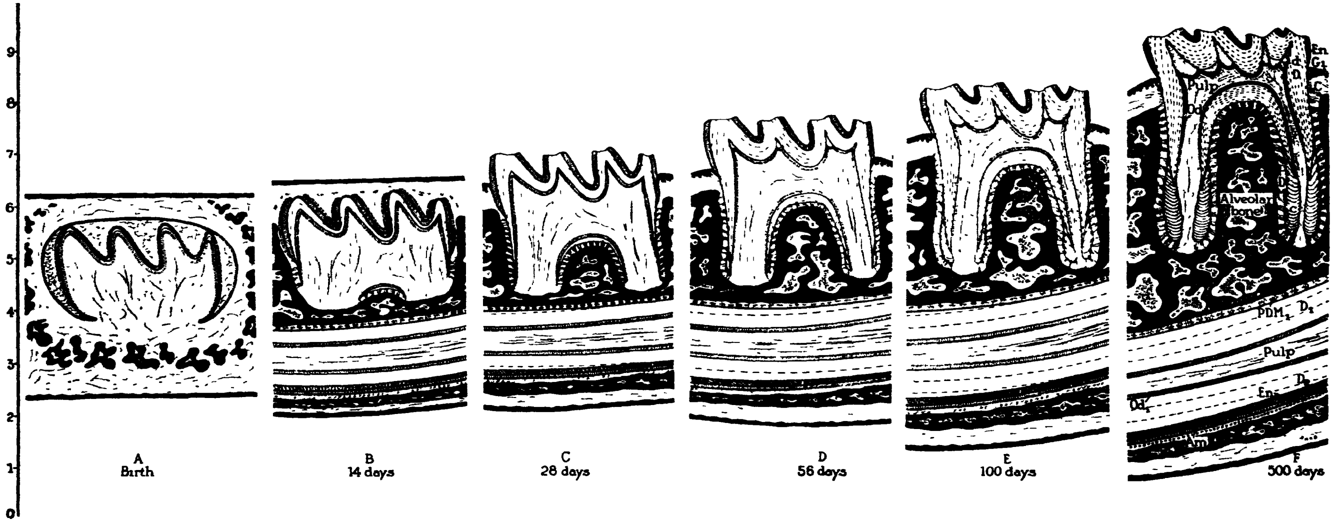


Fig. 71. Diagrammatic representation of the stages in the development of the lower first molar of the rat.

A. Birth. Beginning of appositional growth. The tooth lies unerupted within its bony crypt.

B. 14 days of age. Stage of the completion of the crown. The enamel is completed. Note the enamel free areas over the cusps. Note also the incisor lying beneath the developing lower molar.

C. 28 days. A partly erupted tooth. Formation of the root is just past the root bifurcation.

D. 56 days. A fully erupted tooth showing the increase in the enamel free areas due to attrition, and the formation of secondary cementum.

E. 100 days. Primary dentin formation is nearly completed and the deposition of a large amount of secondary cementum is seen.

F. 500 days. Stage showing changes with age. Note the large amount of attrition, secondary dentin formation at the pulpal horns and secondary cementum formation, coincident with the continuous eruption of the tooth. (From Hoffman and Schour.[20]) En., enamel; Gi., gingvae; D., dentin; d., secondary dentin; C., cementum; P.D.M., periodontal membrane; Od., odontoblasts; Am., ameloblasts.

growth of the tooth but is the result of the lack of wear, just as long hair can be the result not of overgrowth but of lack of trimming. The unworn teeth assume the shape of a typical logarithmic spiral. (Fig. 70.) It may perforate the palate or the lip (Fig. 99) and in wild life will cause the death of the animal through starvation. Since the gradient of growth is very small and the spiral almost a true circle, the elongation has often been described as a perfect circle.[19]

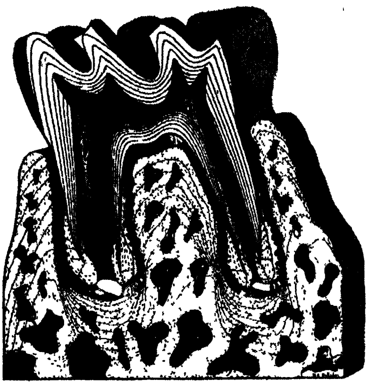


Fig. 72. Diagrammatic representation of a lower first molar of a rat 100 days of age in longitudinal hemisection. Note the incremental pattern in the dentin. (Hoffman and Schour.[20])

Rat Molar

The rat molars undergo the same stages of development as do the incisors but the enamel and dentin are of limited growth.[5, 20]

Growth. The proliferation of the formative organs of the dental tissues and the histo-differentiation of the formative cells are quite similar to that seen in the human molars.[8] However, prior to amelogenesis the enamel organ becomes vascular immediately over the appositional growth centers or future cusps.[21]

TABLE VIII

Anatomy and histophysiology of the incisors and molars of the white rat

	Incisors	Molars
Form	Conical	Molariform
Crown	Covered by enamel on labial; by cementum on lingual	Covered only by enamel Enamel-free cusps
Number of cusps[1]	None	$M_1\frac{5}{4}\ M_2\frac{4}{4}\ M_3\frac{3}{3}$
Roots	Not present	Covered by cementum
Number of roots[1]	None	$M_1\frac{5}{4}\ M_2\frac{4}{3}\ M_3\frac{3}{3}$
Secondary cementum	None	Forms ⅓ or more of root
Growth of enamel and dentin	Continuous	Limited
Growth of periodontal tissues	Continuous and rapid	Continuous and slow
Eruption and attrition	Continuous and very rapid —Upper, 2.1 mm. per week. Lower, 2.8 mm. per week	Continuous and very slow
Length of recording period in the dentin	Last 40–50 days of life	Birth to 125 days

[1] Braikoff [23] Upper and lower molars indicated separately.

As in the human molars, cellular activity begins at definite growth centers (cuspal tips) and proceeds along a rhythmic and regular pattern (Figs. 71 and 72) at definite rates and gradients of growth and for a definite life span of the formative cells (Table VIII). The growth pattern differs only inasmuch as the areas immediately over each growth center (the cusps) are enamel-free. The enamel-free areas are prenatal in origin and are increased in extent by functional attrition.[22]

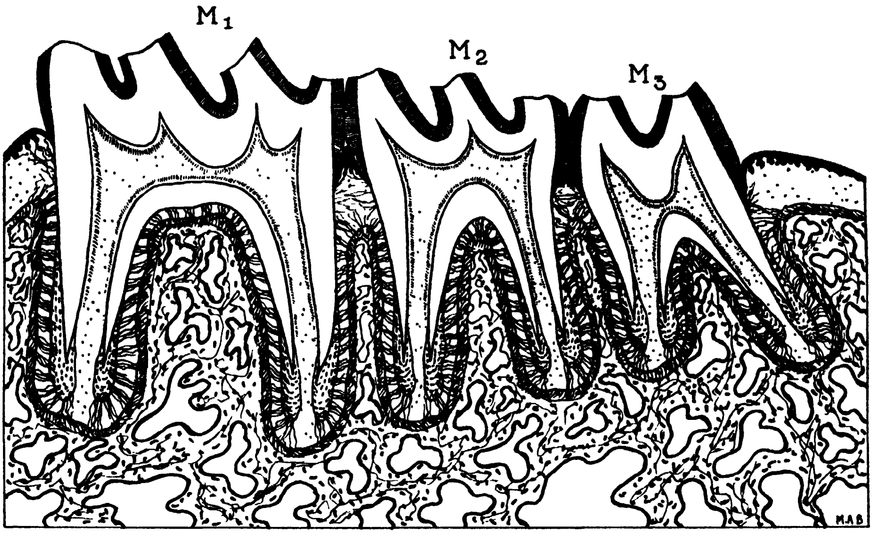


Fig. 73. Diagrammatic representation of the lower first (M_1), second (M_2), and third (M_3) molars of a rat 100 days of age as seen in a midsagittal, anteroposterior section. Note the differences in size and number of cusps, the enamel free areas, occlusal grooves, and the approximal contact areas.

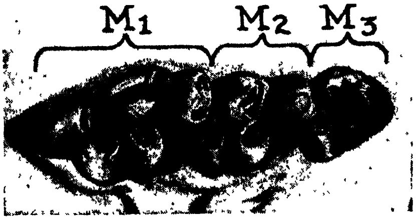


Fig. 74. Occlusal view of molars.

The first molar—which is the largest of the three in each quadrant—begins its formation on the 21st day in utero. The second and third molars begin their formation on the 2nd and 14th days respectively (Table VI).

In the first molar the appositional growth of the enamel is completed at about ten days of age. The enamel organ degenerates and further formation of enamel is therefore not possible. The daily rate of primary dentin apposition ranges from a maximum of 16 μ per 24 hours at the cuspal tips to approximately 2 μ per 24 hours in the roots.[24] The pulp (formative organ of the dentin) remains within the central cavity of the dentin and after the primary dentin is completed, at about 125 days, new pulpal cells differentiate under the stimulus of attrition and form secondary dentin (Fig. 71).

Secondary cementum in the first molar begins to form about the 35th day and is concomitant with the functional stresses imposed upon the teeth, and thus is added continuously throughout the life of the animal, so that in the adult rat one-third or more of the relatively long root is formed by the cementum alone (Figs. 71-74). The roots are 3 to 5 in number (Table VIII).

Calcification. The calcification of the dentin occurs in exactly the same manner as described for the dentin of the rat incisor.

Eruption. The eruption of the molars is continuous throughout the life of the rat, but occurs at such a slow rate as to be normally indiscernible. Prior to the occlusion of the teeth the rate of eruption is very rapid but as soon as antagonism is established the rate is markedly and mechanically retarded. Eruption is accelerated following the loss of an antagonist. The time of eruption of the molars is given in Table VI.

Functional Attrition. Functional attrition is very slow but continuous throughout the life of the animal and is compensated by the continuous, although slow, eruption of the teeth and by the formation of secondary cementum.

Histophysiologic Remarks. Under the stimulus of the slow but continuous attrition of the molar teeth, new dentin-forming cells, cementoblasts, and osteoblasts are differentiated and appose secondary dentin, secondary cementum, and alveolar bone. The growth of these tissues as well as the processes of attrition and eruption are therefore continuous processes.

The essential differences between the anatomy, histogenesis and histophysiology of the rat incisor and the rat molar are given in Table VIII.

The histology and the physiology of the dental tissues of the rat molar are quite similar to those of human molars. In particular, the periodontal tissues approximate closely the human periodontium. The rat molar therefore is a stepping-stone between the rat incisor and the human tooth in evaluating experimental effects upon the teeth. In addition, its form and function are similar to those of the human molars, and lend themselves readily to experimental studies in caries.[25] The conical form and rapid wearing of the incisor is not conducive to caries formation.

The rat molar offers an advantage over the incisor in experimental studies since the experimental record written within the substance of its enamel and dentin remains a long time, while the record in the incisor is lost by the rapid attrition and eruption of that tooth. Thus the weaning ring which reflects the change in nutrition at the weaning period, usually 21 days of age, is readily seen in the molar of the adult rat, while this ring is absent in the incisor of a rat 60 days of age, or older.

METHODS OF ANALYSIS

In studying the effects of experimental conditions upon the teeth of the rat we have adopted the following procedure outlined in Table IX for the gross, radiographic and histologic analyses of the changes.

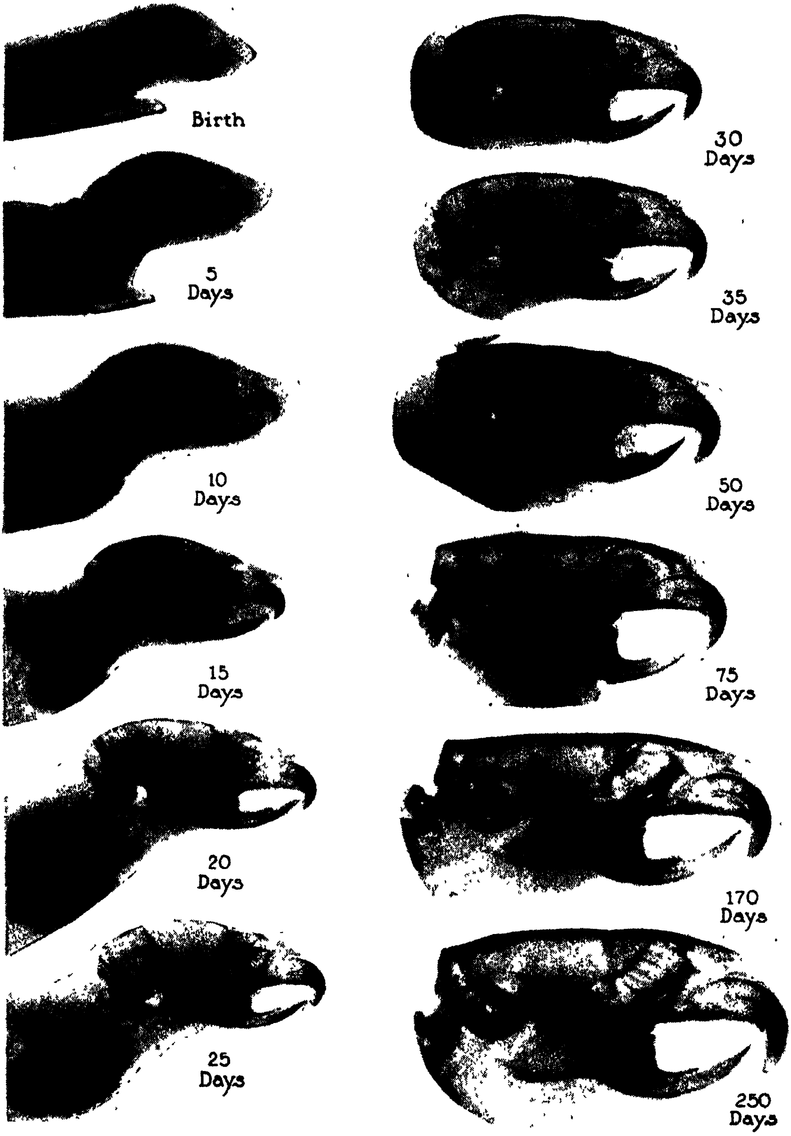


Fig. 75. A series of cephalometric X-rays of the same male rat, from birth to 250 days of age (× 1½). (Spence.[29])

TABLE IX

Methods of analysis of the incisors and molars of the rat

	Gross and Radiographic Analysis	Histologic Analysis
Eruption	(Rate in mm. per week)	Same as under gross and radiographic analysis but in greater detail
	Stage	
Form and contour	Length of clinical crown in 10ths of mm.	
	Mesio-distal and labio-lingual width	
	Dentitional age; Actual age	
	Attrition	
	Fractures, intra- and extra-alveolar	
	(Angle of incisal bevel in degrees)	
	(Incisal relationship)	
	(Arc of spiral in degrees)	
	(Torsions, elongations, proximal buckling)	
	[Length of roots and anatomic crown]	
	[Extent of enamel-free areas]	
	[Caries]	
	In Gross Specimens Only	
Gums and gingivae	Color, tone	
	Height of gingival crest	
	Height of epithelial attachment	
	Depth of gingival crevice. Pocket formations	
	Engorgements, recessions, injections, gingivitis	
	Foreign bodies	
ENAMEL:		
Formation		Odontogenic epithelium
		Enamel organ
		Ameloblasts. Height, cytoplasmic inclusions, atrophy
		Papillary layer. Height, atrophy
	Width in microns	Width in microns
		Enamel-rod arrangement and length in microns
		Rate of apposition in microns per day
	Hypoplasias	Hypoplasias. Types and extent. Time of occurrence
		Organic matrix. Staining reaction
Calcification	Chalky opacities	Incremental stratification, normal or accentuated
	Degree of calcification	Degree of globular fusion

Statements in ( ) apply only to the incisors. Statements in [ ] apply only to the molars.

TABLE IX (*Continued*)

	Gross and Radiographic Analysis	Histologic Analysis
Pigmentation	(Degree)	(Pigment granules in ameloblasts)
Formation	Width in tenths of mm.	Width in microns Dentinal tubules. Arrangement and length Vascular indentations or inclusions
DENTIN:		
Calcification	Degree of calcification Zones of disturbed calcification Fractures	Predentin. Width in microns Interglobular dentin and stratification Time of disturbed calcification Staining reaction. Degree of calcification Mantle dentin Rate of apposition in microns
Pulp	Width in microns and extent Dentifications and cellular inclusions	Width in microns and extent Dentifications, calcospherite formations and cellular invasions Secondary dentin Blood supply Odontoblastic layer. Height, atrophy Degenerative changes. Pulpitis
Cementum	Primary cementum width [Secondary cementum width]	Primary cementum. Width in microns [Secondary cementum. Width] Rate of apposition in microns per day
Periodontal membrane	Width in tenths of mm. (Labial alveolar periosteum) (Basal tissue) Ankylosis	Width in microns 3 layers (cemental fibers, plexus intermedius, alveolar fibers) (Labial alveolar periosteum) (Basal tissue) Ankylosis, fibrosis Vascularity Epithelial rests
Alveolar bone	Level of alveolar crest Degree of calcification Lamina dura (Basal bone width) Spatial relationship to other teeth and to jawbone Periapical involvements	Level of alveolar crest Basal bone width in microns Distribution and character of bone marrow and trabeculations Resorptions and osteoclastic activity Rate of apposition in microns per day Osteoid border width Staining reaction. Degree of calcification Periapical involvements

TABLE X

Developmental physiology of the tooth

Phases in Development	Physiologic Characteristics	Sensitivity to Experimental Interferences	Methods of Measuring
I. Growth a. Proliferation	Cell Multiplication	Low	Number of Mitotic Figures
b. Histodifferentiation	Cell differentiation and assumption of definitive form plan (e.g., outlining form of tooth)	More	Cytologic changes in and positioning of cells
c. Apposition	Secretion and deposition of matrix by formative cells	Most sensitive phase in growth	Vital Injections (Alizarine, NaF, Trypan Blue, etc.)
II. Calcification	Precipitation of calcium salts in protein matrix Follows apposition in close succession Enamel and Dentin, unlike Bone, are not subject to calcium withdrawal	Most sensitive phase in development; sensitive to even normal variation in body metabolism. Reflects constitutional status of individual (Neonatal adjustment, weaning, infancy, etc.)	a X-ray Densiometer. b. Histologic staining reaction c. Degree of fusion of calcospherites d. Vital injections of Alizarine
III. Eruption	Extrusion of the formed and calcified tooth into the oral cavity by a force acting at its basal end. Apposed by incisal or occlusal stresses so that the eruption potential is never fully expressed	Very easily affected by local factors. Less readily affected by systemic factors	Marking of tooth by notching
IV. Attrition	Result of functional wear. Compensated by continuous eruption of tooth and growth of periodontal tissues	?	Marking of tooth by notching

Gross Analysis. For the experimenter who is interested primarily in organs other than the teeth, a gross analysis of the dental changes is still valuable

TABLE X (*Continued*)

	Rate of Activity	Experimental Interferences Result in	Experimental Conditions Showing Specific Disturbances
Ia.	?	Disturbances of all subsequent phases of growth	Colchicine and Lead Poisoning: (deficient proliferative growth) Hypovitaminosis A: uninhibited proliferation of enamel epithelium and invasion into pulp
b.	?	Irregular form of tooth and atypical structure of matrix formation	Hypovitaminosis A: (Atypical dentin formation and form of pulp)
c.	Enamel and Dentin of: Incisors: 16 μ/24 hrs. Molars: Grades evenly from 16μ to 1.2 μ/24hr. Alv. Bone: Av. 4 μ/24 hr.	Hypoplasia (deficient amount of matrix formation)	Hypovitaminosis A: (markedly disturbed gradient of growth. Enamel hypoplasias and vascular inclusions in dentin)
II	Same as rate of apposition (recurs in rhythmic increments of 16 μ)	Enamel: Immature and acid resistant, chalky and fragile Dentin: Nonfusion of calcospherites (interglobular and fragile dentin), wide predentin Cementum and Bone: Wide osteoid and precementum borders	Hypovitaminosis D: (wide predentin and interglobular dentin) Parathyroidectomy: (interglobular dentin) Fluorosis: (chalky-white opacity of enamel)
III.	Upper Incisors: 2.1 mm. per week Lower Incisors: 2.8 mm. per week Molars: Very slow	Deficient Eruption: Submersion Excessive Eruption: Elongation (supraocclusion) Perverted Eruption: Malposition of the tooth	Local: Traumatic occlusion causes decreased rate of eruption Removal or fracture of antagonists allows for increased rate of eruption Systemic: Hypophysectomy, thyroidectomy. Decreased rate and final cessation of eruption
IV.	Equal to rate of eruption	Deficient Attrition: Elongation (supraocclusion) Excessive Attrition: Abnormal wearing of crown	Local: ? Systemic: Deficient calcification of enamel (fluorosis) results in abnormal attrition

since the teeth are very sensitive biologic indicators and will often show experimental effects when other organs fail to do so. Erdheim,[6] for example, was able to select his rachitic animals on the basis of the opaque appearance of the incisors. Moreover, the first recognition of a relationship between calcium metabolism and the parathyroids came from the gross observation of so-called spontaneous fractures of the incisors of parathyroidectomized rats.[26]

Such obvious findings in the incisors as elongation (Fig. 70) due to nonattrition, fractures, lack of pigmentation (Fig. 87), loss of translucency (Fig. 87), form and contour (Fig. 99), can be readily assessed in a few moments' time and even with little experience. In the molars the degree of attrition, gingival recession, fractures of cusps, and caries can be evaluated with the use of a hand lens.

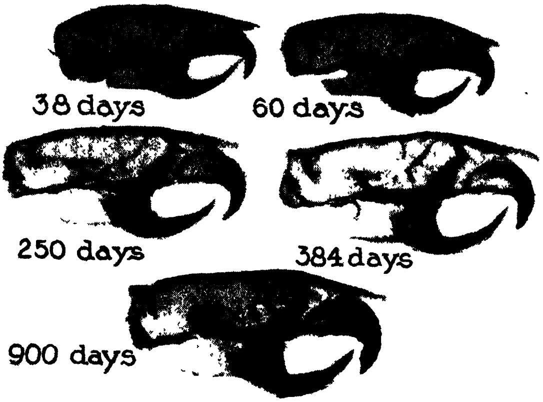


Fig. 76. A series of x-rays of the heads of female rats of the Experimental Wistar Stock (three-fourths natural size). (Courtesy of The Wistar Institute.)

For those interested primarily in the dental changes incident to their experimental procedure this examination must be supplemented by a more detailed and exacting analysis and by a radiographic and histologic study. When the experimenter is unable because of lack of time or experience to make a detailed dental analysis, collaboration with a dental investigator is indicated.

Radiographic Analysis. Lateral x-rays can be taken of the experimental animal after its sacrifice by cutting the head through the midline with a thin saw or razor blade. Each half can then be placed flat upon an occlusal dental film. A standardized exposure time and distance from the x-ray tube should always be used in addition with a penetrometer so that the x-rays are comparable and the comparisons are valid.[27]

In the living animal the cranial radiographic technic and apparatus as employed by Burr and Mortimer[28] or Spence[29] can be used to trace the experimental effects in the same animal serially over a period of time. A radiographic analysis is a valuable supplement to the gross and histologic analysis and provides an objective and permanent record. With the aid of a hand lens or dissecting microscope a quantitative and therefore more exact analysis can be made. The width of the dentin, and pulp (as well as the degree of calcification) can be measured easily (Fig. 99). The form and contour, fractures, and hypoplasias can be analyzed more readily in the radiograph than by gross study. The width of the periodontal membrane and the growth of the alveolar bone can also be evaluated.

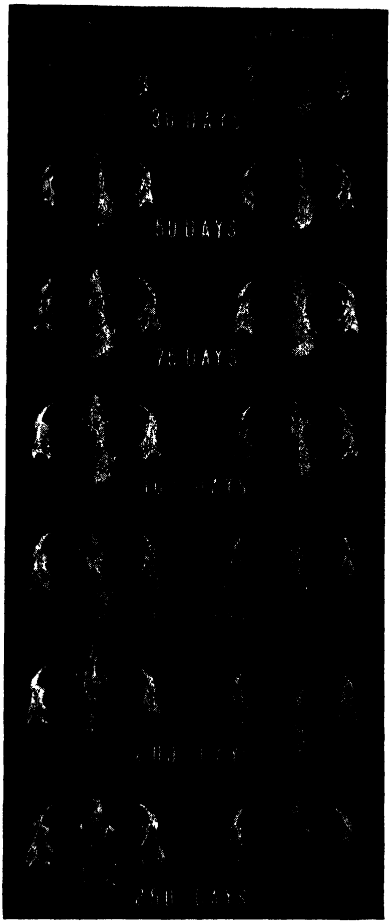


Fig. 77. Skulls of rats at various ages. (Courtesy Dr. E. J. Farris.)

Histologic Preparation and Analysis. The technic for the preparation of decalcified sections can be found in any standard text of histologic technic.[80]

When the analysis of soft tissues is of no concern, ground sections offer a simpler and often a more advantageous method of histologic analysis. In ground sections, the calcified enamel is retained for examination and a section can be prepared in a few minutes with no special or expensive equipment. In addition, in animals injected with alizarine Red S the effects are not destroyed by the decalcifying acid. A small saw or cutting disk, a coarse stone for grinding, and a fine one for finishing are sufficient. Greater speed is obtained when the stones are mounted on a motor. The incisor is dissected either with or without the surrounding bone. Transverse sections can be cut in slabs 1 mm. thick with a fine saw or disk. For longitudinal or transverse sections, the tooth is ground on one side to the desired plane on the coarse stone and finished on the fine stone. The section is then reversed and ground and finished to the desired thickness (about 30 μ). The entire process takes about three minutes. The grinding may be done with the finger or a cork. In the case of large sections or when the dental investing apparatus is to be kept intact, the preparation, after the first plane is finished, may be mounted in any common cellulose cement.

Cox and Dixon[31] have developed an ingenious method of grinding successive parallel planes of rat molars which are examined by reflected light.

Biochemical Analysis. Different methods have been used for the determination of water, organic matter, and ash,[32] of calcium,[38] of magnesium,[34, 35] of phosphorus,[36] and of phosphatase.[37] Matsuda[38] gives the chemical findings of ash, organic matter, water, calcium, phosphorus, and magnesium in the incisor teeth of the male from 23-150 days of age. Smith and Lantz[39] analyzed the phosphatase content of the incisors of rats placed on a fluorine diet and of control rats.

A more recent method of biochemical analysis consists of the special marking of particular ions. In the case of phosphorus the radioactive isotope can be used as a method of vital "staining" and the metabolism of inorganic phosphorus can thus be accurately traced. This has been done in the bones and the teeth of the rat by Hevesy[40] and Manly and Bale.[41] If a quantitative chemical test for the calcium salt of alizarine Red S could be developed the analysis of the metabolism of inorganic calcium would be facilitated.

METHODS OF MEASURING

> The study of form may be descriptive merely, or it may become analytical. We begin by describing the shape of an object in the simple words of common speech: we end by defining it in the precise language of mathematics; and the one method tends to follow the other in strict scientific order and historical continuity.
>
> D'Arcy Thompson: Growth and Form. p. 719.

Every biologic fact and process should have a quantitative basis. The normal processes and the deviations from them should not merely be described but should also be evaluated quantitatively. A quantitative discipline serves to remove the subjective faults from the analysis and to make the findings valid for comparison with the findings of others. It may lead to the discovery of more far-reaching laws than is possible by simple description.

Each method of analysis should whenever possible be designed to measure specifically the developmental process under consideration (growth, calcification, eruption, and attrition). A comprehensive study of development should therefore include different methods of analysis, each designed to elucidate the findings of a particular process.

Measurements of Growth

The Rate of Appositional Growth. We have employed the method of vital staining for measuring the rate of appositional growth. Intraperitoneal injections of alizarine Red S (Sodium Sulphalizarate) (100 mg. per Kg. in an isotonic 2% solution) gives a red coloration to the dentin, cementum, and the bone that is forming and calcifying at the time of the injection (Fig. 78). In this way a sharp red line is produced which can be seen readily in ground sections.

The method of vital staining with alizarine Red S has been very useful in the analysis of the growth pattern of dental structures as well as bone. When the injections are given at sufficiently wide intervals the effects can be seen with the naked eye or under low magnification, so that microscopic preparations are not necessary.

It would be of tremendous advantage to so modify the alizarine solution that it would be acid resistant and permit the preparation of decalcified serial sections. Even more valuable would be the preparation of an alizarine solution that would be radiopaque in its effects. It would then be possible to study growth and calcification in the living animal without the necessity of sacrificing that animal.

Another method of vital staining consists of intraperitoneal injections of 0.5 cc. of a 2.5% solution of sodium fluoride. This produces a sharp and distinct accentuation of the incremental line in the enamel and dentin forming and calcifying at that time (Figs. 79-80). The effect can be recognized in decalcified as well as in ground sections. Other methods of vital staining have been employed (trypan blue,[42] uroporphyrin,[43] and hematoporphyrin[44]).

By the method of vital staining the sites of growth are clearly demarcated and a chronologic landmark is experimentally produced in the hard tissues. The rate of apposition can be quantitatively measured by multiple injections. The distance between two successive injection lines divided by the intervening number of days will give the daily rate of apposition.[45]

The normal rate of apposition of the enamel and dentin in the incisor teeth is approximately 16 μ per 24 hours. The rate in the molar grades from a maximum of 16 μ to a minimum of 2 μ per day.[24] The daily rates of apposition of the alveolar bone vary according to age and location.[20]

The Growth Potential of the Formative Cells. When the growth potential of the formative cells is expressed, the final length of the enamel rod or the dentinal tubule is reached. The length of the latter is the product of the rate of apposition and the active life-span of the formative cells. The life-span may thus be obtained by dividing the length of the enamel rod or dentinal tubule by the average daily rate of formation.

Hypoplasias. Enamel hypoplasia and vascular inclusions in the dentin are the result of a sudden cessation in cellular activity. They usually are associated with severe local or systemic disturbances that kill the formative cells before their normal life-span is completed. The location of the resulting defect in the enamel or dentin indicates the age of the cells and the chronologic level

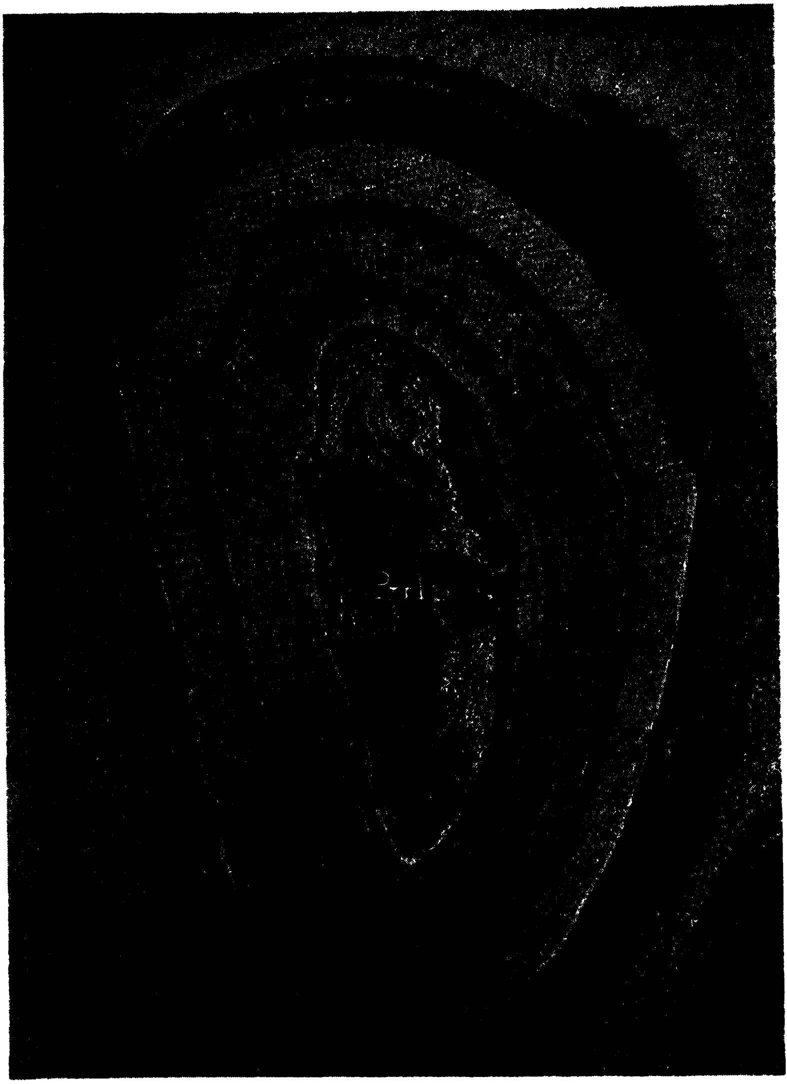


Fig. 78. Photomicrograph of a transverse ground section of the lower incisor of a rat that was given two injections of alizarine Red S on the 42nd (1) and 52nd (2) day of age and sacrificed on 56th day (× 75). The effects of the injections are seen in the dentin as concentric red incremental rings 160 microns apart. The dosage in the second injection was much smaller than in the first. Note the difference in intensity of the vital staining. Distance of the line from the pulp divided by 16 μ (rate of apposition) indicates the number of days before death that the injection was given. Al. B., alveolar bone; 1′, effect of first injection in the alveolar bone.

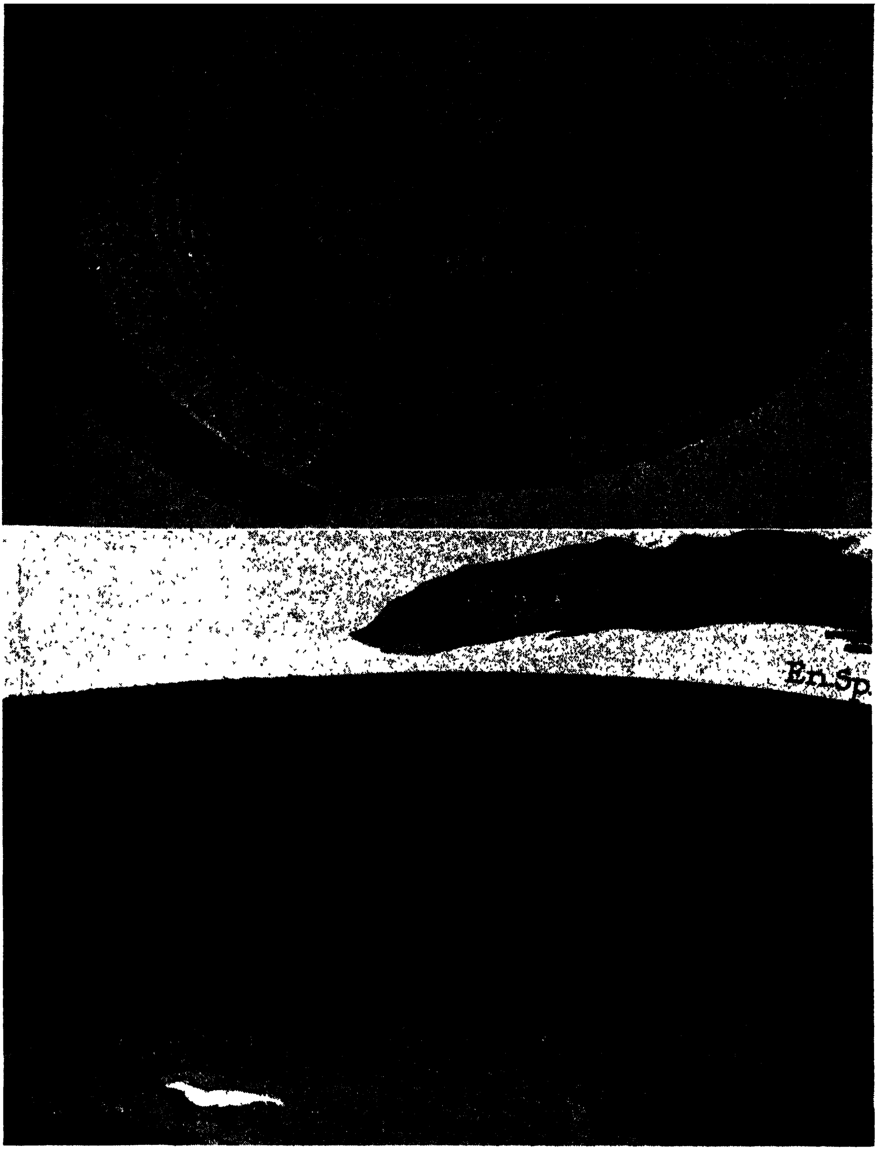


Fig. 79. (A) Photomicrograph of a transverse ground section of the lower incisor of a rat which was given four injections of sodium fluoride at 48-hour intervals and killed 14 days later (× 57). The effects of the injection are seen in the dentin as concentric incremental stripes of disturbed calcification 32 μ apart. D_1, dentin formed before the experiment; D_2, dentin formed during the experiment. The stratification in the dentin formed after the experiment (D_3) consists of fourteen daily pairs of dark and light stripes, which correspond with the days elapsing between the last injection and death. (Schour and Smith.[75, 76])

(B) Photomicrograph of a decalcified section of the upper incisor of the same rat as in A showing the labial dentin at the level of the gingival crest. En. Sp., enamel space; Od., odontoblasts. (From Schour and Smith.[75, 76])

at which the disturbance has occurred. The extent of the lesion, in some instances, indicates the severity of the disturbance.

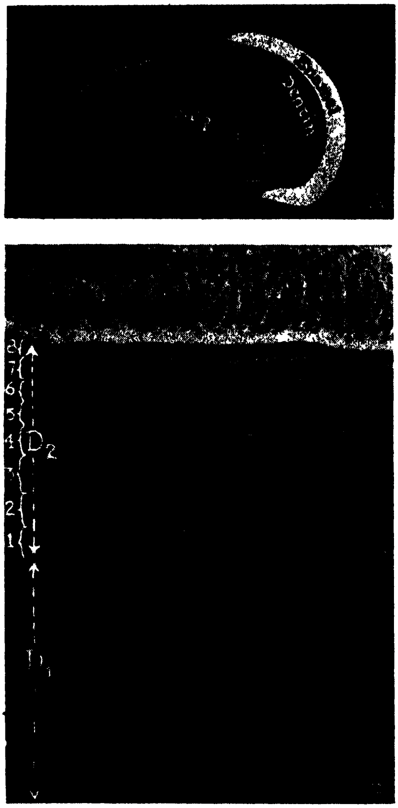


Fig. 80. (A) Grenz Ray of a transverse ground section of the upper incisor of a rat that was given 8 injections of sodium fluoride at 48-hour intervals and killed 48 hours later (× 8). The effects of the injection are seen in the dentin as concentric radiopaque incremental stripes approximately 32 micra apart. (The Grenz Ray was kindly prepared by Dr. E. Applebaum.) (From Schour.[9])

(B) Decalcified section of a corresponding area indicated in A (× 78). D_1, dentin formed before injections were given. Note interglobular dentin due to suboptimal vitamin D content in the basal diet. D_2, dentin formed during period of 8 injections of sodium fluoride. Od., odontoblasts; Pre., predentin. (From Schour and Smith.[75, 76])

Measurements of Calcification

It has been difficult to evaluate the degree of calcification on a quantitative basis. However, a few general methods have been utilized:

1. The densiometric x-ray (Van Huysen, Bale, and Hodge).[46]
2. The Grenz ray (Fig. 80) (Applebaum, Hollander, and Bödecker).[47]
3. Polarized light (Kitchin).[48]

4. Diffraction studies (Reynolds et al).[49]
5. Microhardness (Karlström, Hodge).[50, 51]
6. Microincineration (Hampp).[52]
7. Chemical analysis.[38]
8. Histologic analysis.[30]

Histologic Analysis. The histologic method of studying the calcification of the tooth, particularly the dentin, and bone is simpler than the other methods and has been the most useful and satisfactory one in this laboratory. The histologic appearance of teeth is a more sensitive index of dietary effects on calcification than the chemical analysis.[53, 54, 55] The following factors should be considered:

The Width of the Predentin (and the Osteoid Border). Normally the width is 10-20 μ. The increased width of the predentin in some cases (rickets) is directly proportional to the degree of disturbance in calcification.[56] In disturbed calcification, the intermediate-dentin border is usually wavy.

The Degree of Globular Fusion in the Dentin. Normally the fusion of the globules is complete. As the disturbance in calcification increases, the degree of fusion decreases so that interglobular dentin results. The different degrees of globular fusion are illustrated in Figures 68 and 69. The enamel is more sensitive to disturbances in calcification than the dentin but is also less expressive. In both, an accentuation of the normal incremental stratification indicates a sub-clinical disturbance in calcification.

Hypoplasia. Although enamel hypoplasia and vascular inclusions in the dentin are manifestations of growth disturbances involving an arrest in cellular activity, usually they are associated with systemic disturbances in calcium metabolism. It seems that relatively mild disturbances in calcium metabolism are reflected in a disturbed calcification of the hard tissues, but if the systemic effect is sufficiently severe, cellular activity may be affected as well and hypoplasias result.

Staining Reaction. In decalcified sections the hard tissue that stains with hematoxylin is considered to have been well calcified and the eosin-staining tissue is considered to have been relatively less calcified. It is fully realized that this interpretation is empiric but nevertheless useful because it does indicate basophilic and acidophilic reactions and has been found to be reliable.[57] We have used other dyes such as alizarine Red S, silver nitrate and Mallory's triple connective-tissue stain in a similar manner and with similar results. In studying the calcification of enamel matrix, Mallory's connective-tissue stain is especially valuable because it stains differentially the young, primary, and transitional stages in the progressive calcification of the organic enamel matrix.[58] (Fig. 69.)

The rate and the time of the calcification process may be analyzed by the same methods employed in obtaining the time and rate of appositional growth, since calcification follows apposition in close succession.

Measurement of Eruption and Attrition

In the adult rat the rate of attrition is normally compensated by an equal rate of eruption, so that the tooth remains relatively constant in length. The rate of eruption is readily measured by marking the exposed enamel surface at the gingival line with a fine saw, jeweler's file, or lightning strip. These markings can be made upon the incisor without anesthetizing the rat. Shadle and Skarupinski[59] have devised a zipper tube for holding the rat during such operations, but the rat can be held in one's hand without difficulty.

The migration of the notch from the gingival crest indicates quantitatively the rate of eruption of the tooth. This distance can be measured with an adjustable caliper. The normal rate of eruption is 2.1 mm. for the upper and 2.8 mm. for the lower incisor.[2, 15] The decrease in distance between the notch and the incisal edge is a measure of the rate of attrition.

In the molars the rate of eruption and attrition cannot be measured directly because of the difficulty of access to these teeth in the living animal. However, if a radiographic technique is employed, the cemento-enamel junction may be employed as the "notch" or fixed point on the tooth and its migration in relation to the alveolar crest may be used to measure eruption. Similarly, the distance of the cemento-enamel junction from the occlusal surface may be used to measure attrition. For stages in eruption the reader is referred to Noyes, Schour, and Noyes.[8]

Pigmentation

Although pigmentation of the enamel is characteristic of the rat incisor and does not occur in the rat molars, it does offer another method of evaluating experimental effects upon the teeth. The pigmentation is first seen in the rat four weeks of age, and is of a yellow-orange color which deepens with age. The pigment covers the anterior two-thirds of the enamel in the upper and the anterior half in the lower incisor. Although the significance of such findings is still obscure, changes in pigmentation are often the earliest demonstrable manifestation in various experimental interferences (see fluorosis). A useful colorimetric chart has been prepared and used by Hodge, Luce-Clausen, and Brown to evaluate the degree of pigmentation in the incisors.[60]

Apparently the pigment is of inorganic nature, since it is retained and accentuated following microincineration.[61] M. C. Smith observed an absence or diminution of pigment in the incisors of anemic rats. The iron content of the pigment merits investigation.

EXPERIMENTAL EFFECTS ON TEETH

Growing and Non-growing Structures. In attempting to analyze the response of the dental tissues to systemic and local interferences, we must carefully distinguish between the growing and the non-growing structures. The biologic reaction of tissue depends upon the fact that while it is in the process of development the formative cells can be affected by local or systemic factors and such disturbances are automatically reflected and recorded in the growing structure. On the other hand, after growth and development are completed. the formative cells are quiescent, non-active or even degenerated and lost. Therefore, a local or systemic disturbance will leave no record since there are no active cells present to be affected and no growing structure to record such effects.

All structures are formed by cells with a definitely limited life-span. When that life-span is expressed, the structure is completed. But in certain situations new cells constantly replace the old and the growth and development of that structure is continuous. Thus, in the rat incisor enamel and dentin are continuously being apposed by cells that are constantly being derived from a continuously proliferating odontogenic epithelium. In the rat molar, on the other hand, the odontogenic epithelium is limited in its activity and no new cells replace the old, and in that sense the growth of the tooth is limited.

The Kymographic Quality of the Growing Tissues. All growing tissues are affected in their development by local or systemic disturbances and record these effects within their substance by deviations from the normal structure. Thus each growing tissue in the body acts like a kymographic record in registering experimental effects. If the tissue, like enamel and dentin, is a permanent one, the record will also be permanent. But if the tissue undergoes constant regeneration and replacement, such records are transitory.

In the incisors of the rat the kymographic record rolls on throughout the life of the animal with relative rapidity and permits a ready reading of the metabolic events of the current fifty days of life. However, the record of the preceding period is lost by the rapid wear of the tooth in function. In the rat molar, where the wear is slow, the constitutional record of the first 125 days of life of the animal is available for permanent reference. Thus a study of both the incisors and molars permits an analysis of the complete or major part of the life history of a given animal. In the bone, a continuous record is also obtained since the old bone is constantly being resorbed and replaced by newly forming tissue. Such records are, in turn, quickly lost by resorptions and repairs. This is particularly true of the alveolar bone and the other periodontal tissues which are continuously being regenerated or apposed by newly active periodontal cells, under the stimulus of continual function of the tooth. In histologic analysis of experimental effects upon the teeth these fundamental considerations must always be borne in mind, and the recordings should be evaluated accordingly. (See plate facing title page.)

The influence of the different vitamins or endocrines upon the teeth is so characteristic that in many instances it is possible, by a radiographic or histologic examination of the teeth, to diagnose the experimental condition without the knowledge of the laboratory records.

The gross, radiographic, and histologic alterations in the growth, calcification, and eruption of the growing teeth that characterize given experimental interferences are of special significance in so far as they reflect and point out disturbances in the physiologic processes of the animal as a whole. These disturbances may affect cells and tissues in other parts of the body in a similar manner.

EFFECTS OF THE VITAMINS ON THE TEETH

Hypovitaminosis A

Growth. Vitamin-A deficiency in rats produces severe alterations in the proliferative, differential, and appositional phases of the growth of the teeth. The earliest changes which can be recognized are disturbances in histo-differentiation. The odontoblasts fail to differentiate normally and the proliferative growth of the odontogenic epithelium, which normally stops when differentiation begins, persists. As a result, epithelial cords derived from the odontogenic epithelium invade the pulp (Fig. 81). Here they sometimes stimulate the pulpal mesenchyme to form an atypical dentin (Fig. 81).

The rate of dentin apposition is markedly altered. The early physiologic differences between the labial and lingual portions of the incisors seen at birth are now strikingly accentuated. It appears that the cementum-covered dentin is constitutionally inferior to the enamel-covered dentin. In vitamin-A deficiency the latter shows an accelerated rate (up to 19 μ per day) and the cementum-covered dentin shows a decelerated rate (down to 6 μ per day) in a uniform gradient up to 310%. (Fig. 82.) The fact that the extent of the deviation from the normal gradient (10%) is in direct proportion to the severity and duration of the vitamin-A deficiency suggests the possible use of this sensitive dentin reaction as a biologic method of measuring the vitamin-A content of foods.[63] The findings indicate that the dentin of the rat incisor acts as a growth kymograph as well as a kymograph of mineral metabolism.

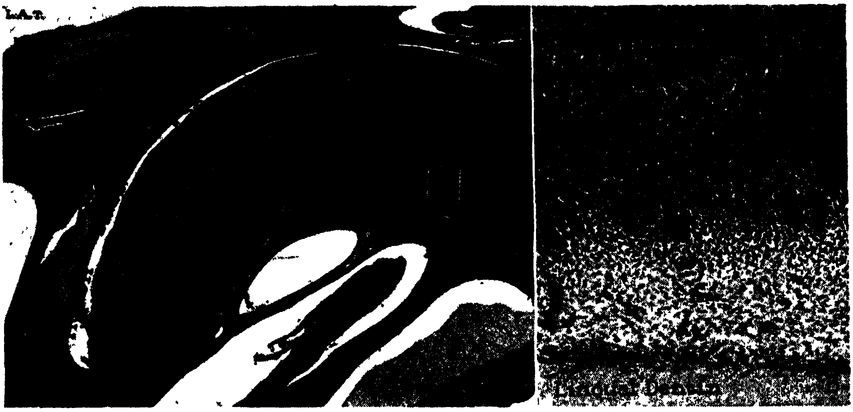


Fig. 81. (A) A longitudinal section of the basal one-half of an upper incisor of a rat fed a diet deficient in vitamin A (× 9). Note the abnormally thin lingual dentin (D), the invasion of the pulp by the epithelial cords (Ep. C.) from the odontogenic epithelium (Od. Ep.); osteodentin (Os. D.), and hypoplastic enamel (Hyp. En.). En. Ep., enamel epithelium; E. Sp., enamel space; Id., interglobular dentin; L.A.P., labial alveolar periosteum; L.B., labial alveolar bone. (From Schour.[45])

(B) Higher magnification of corresponding region indicated in figure A (× 77). Note the rich amount of epithelial proliferation and cords in the pulp and the amorphous structure of the lingual dentin. Un. Od., undifferentiated odontoblasts. (From Schour, Smith, and Hoffman, Amer. Jour. of Path., 1941.)

The enamel is hypoplastic. The presence of vitamin A is essential to the differential growth of the odontogenic epithelium and of the formative cells, and thus is essential to the entire process of tooth formation.[10, 64] A long-continued chronic vitamin-A deficiency produces tumor growths (odontomata) which include imperfect germinal tooth-bud structures in and about the incisor teeth.[65]

Calcification. Calcification is affected, but relatively less than is growth. The dentin shows an interglobular texture. The alizarine lines take on a faint red color as compared with the normal vital staining of deep red.

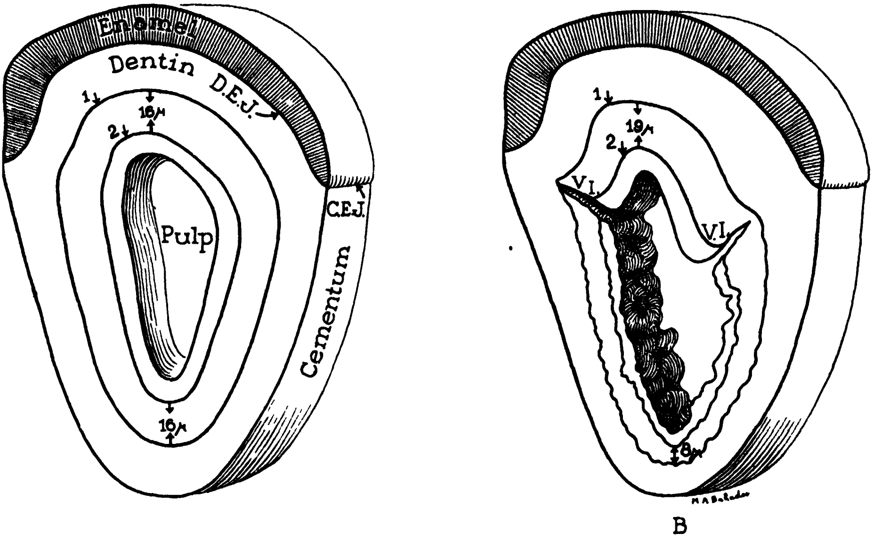


Fig. 82. Semi-diagrammatic tracing of a transverse section of the lower incisor of a normal (A) and vitamin-A deficient animal (B). The lines 1 and 2 in the dentin represent the red lines resulting from alizarine injections given 10 days apart. The animal was sacrificed 5 days later. Note the effect of vitamin-A deficiency upon the gradient of growth. The normal rate of apposition (16 μ per day) is increased in the enamel-covered dentin to 19 μ and decreased in the cementum-covered dentin to 8 μ. Note the distortion of the pulpal outline and the vascular inclusions in the dentin (V.I.) which are most severe at the cemento-enamel junction (C.E.J.). D.E.J., dentino-enamel junction. (From Schour, Smith, and Hoffman.[63])

Smith and Lantz[66] found a lower percentage of ash but a higher percentage of calcium and a slightly lower percent of phosphorus in the ash in the incisors of rats fed a diet deficient in vitamin A.

Eruption. Eruption is progressively retarded.[67]

Periodontal Tissues. Mellanby[4] has described an increased density and fewer lacunae in the alveolar bone.

Pohto reports negative dental changes in hypervitaminosis A.[68]

Hypovitaminosis C

While the dental changes in scurvy have been repeatedly studied in the guinea pig and have been used as a biologic method for the assay of vitamin C,[69] little is known of the influence of vitamin C on the teeth of the rat. It is difficult to produce vitamin-C deficiency in rats, probably because they synthesize sufficient vitamin C for their adequate nutrition. Westin and Kalnins[70] report severe disturbances in the periodontal tissues of both the incisors and molars of rats placed on a basal diet free of vitamin C.

Hypovitaminosis D

One of the earliest gross manifestations of rickets is the loss of pigmentation and transparency of the exposed portion of the incisors. In addition, calcification is markedly affected whereas growth is relatively undisturbed, so that an experimental dissociation between calcification and growth is produced.

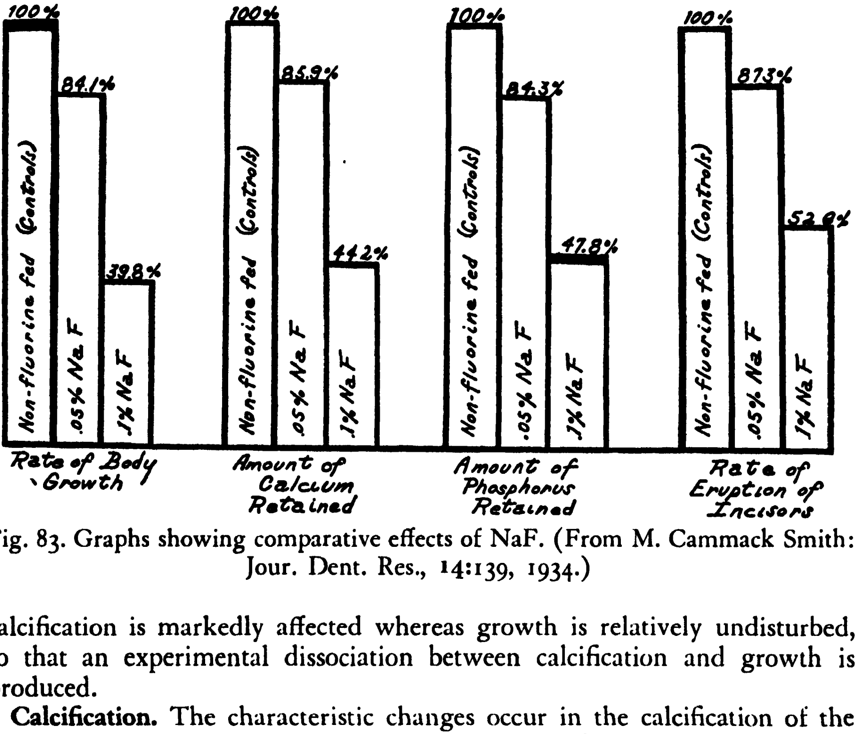


Fig. 83. Graphs showing comparative effects of NaF. (From M. Cammack Smith: Jour. Dent. Res., 14:139, 1934.)

Calcification. The characteristic changes occur in the calcification of the hard dental tissue, particularly the dentin. Erdheim[6] in his classic work on "Rickets and Parathyroids" characterized the dentin reaction for rickets in terms of the following three zones traced from the pulp outward: (1) wide predentin; (2) transitional zone of varying degrees of imperfect interglobular calcification; (3) outer zone of well-calcified dentin next to the dentinoenamel junction (mantle dentin) (Fig. 83, and Frontispiece). Some of these changes can be seen in the roentgenograms (Fig. 99).

The delicacy of the dentin response to even slight vitamin-D deficiency can be illustrated by the following experience: In a collaborative study, normal control animals which were sent to the laboratory showed a dentin of interglobular texture beyond normal range. This was disconcerting because adequate normal controls are essential to every experimental investigation. The vitamin-D content of the basal diet was suspected and upon investigation it was found that it depended on whole milk for the sole source of vitamin D. The fortification of this diet with a few drops of irradiated ergosterol corrected the deficient calcification of the dentin. Thus, using the dentin as an indicator it was found that the vitamin-D content in whole milk was not sufficient for this group of animals. It is not uncommon to find the basal diet of various laboratories containing suboptimal amounts of vitamin D (Fig. 60).

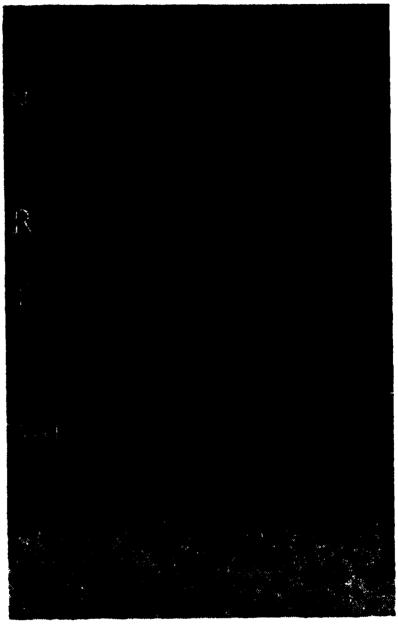


Fig. 84. Photomicrograph of a section of labial dentin of the lower incisor of a rat given 0.5 cc. of irradiated ergosterol 10,000 X and killed 113 hours later (× 200). N., dentin laid down and calcified before the experiment; R., dentin calcified during the rise of the blood calcium (hypocalcified) F., dentin calcified during the fall of the blood calcium (hypercalcified). Od., odontoblasts; P., predentin. (From Schour and Ham.[57])

Becks and Ryder[71] observed odontoblastic atrophy which they regarded as a primary effect of rickets (Fig. 88). However, this finding is not constant.

Growth and Periodontal Tissues. A hyperplasia characterized by a wide osteoid border occurs in the alveolar bone, especially in the basal region. In the molars this hyperplasia often leads to a bridging of the periodontal membrane.[71] A very wide precementum border is also present.

Eruption. Changes in eruption have not been conclusively established.

Hypervitaminosis D

Calcification. The effects of administrations of high doses of irradiated ergosterol on the dentin of the rat incisor are similar to those obtained from injections of parathyroid extract; namely, a primary reaction in the form of a poorly calcified dentin layer that is related to the rise of the blood calcium and a secondary reaction in the form of a well or excessively calcified dentin layer which is related to the return of the blood calcium to normal.[57] (Figs. 84 and 85.)

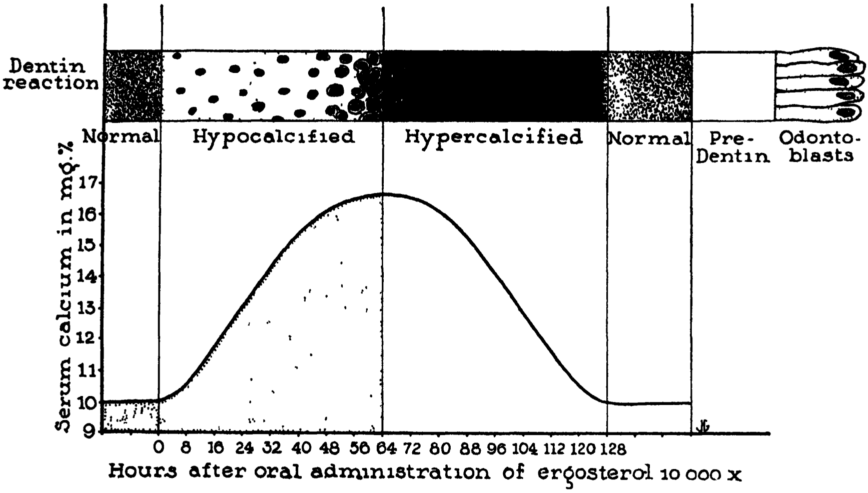


Fig. 85. Graph showing rise and fall in serum calcium after a single dose of irradiated ergosterol, and a diagrammatic representation of the concomitant calcification in the dentin. (After Schour and Ham.[57])

While the influence of vitamin D upon the calcification of the teeth can hardly be questioned, the mechanism of its action presents unsolved problems for research.

Periodontal Tissues. The alveolar bone shows hypercalcification and in the molars the cementum is hyperplastic as well as hypercalcified so that a partial ankylosis of the periodontal membrane results.[72, 73, 74] The histologic findings vary with the age of the animal, the dosage, and the duration of the experiment. The changes in growth and eruption have not yet been conclusively investigated.

EFFECTS OF INORGANIC SALTS ON THE TEETH

Fluorosis

The growing tooth is the first organ to react to fluorides and the growing enamel and enamel-forming cells give the most delicate response.

Acute Fluorosis. The earliest effect of an intraperitoneal injection of small doses of 0.3 cc. of 2.5% sodium fluoride in rats is a disturbance in the ameloblasts which can be recognized a few hours after an injection.[75, 76, 77] (Fig. 86.) The cytoplasm shows an abnormal character and disturbance of its globules. This reaction suggests that fluorine exerts a direct local action on

TABLE XI

Summary of the effects of diet upon the tooth development

Experimental Condition	Growth	Calcification (of Hard Tissues)	Eruption	Pathognomonic Syndrome
Hypovitaminosis A	Proliferation and graded appositional growth (histo-differentiation) markedly disturbed in the dentin.	Deficient (interglobular dentin).	Retarded	Disturbed epithelial proliferation. Histo-differentiation and gradient of appositional growth.
Hypovitaminosis D	Not disturbed except for a hyperplasia of osteoid and precementum borders with ankylosis.	Markedly deficient (interglobular dentin and wide predentin, osteoid and precementum borders).		Deficient calcification with relatively undisturbed appositional growth.
Hypervitaminosis D	*	Acute primary hypocalcification followed by secondary hypercalcification of dentin. Hypercalcification of alveolar bone and cementum with ankylosis.	*	Acute primary hypocalcification, secondary hypercalcification. Concomitant rise and fall in blood-calcium level.
Fluorosis	Ameloblasts occasionally injured.	Deficient calcification of enamel and dentin.	Slight retardation in proportion to dosage.	Disturbed calcification by direct chemical action on cells.

* Data insufficient. The effects of hypervitaminosis A, vitamin-B complex, vitamin C, vitamin E, total and individual salt deficiencies (i e , magnesium, calcium, phosphorus, etc.), and the essential amino acids upon the teeth merit further investigation

the enamel-forming cells. The surface of the organic matrix is covered with globules which stain deeply with hematoxylin (Fig. 86).

Calcification. When the tissues are examined 24 hours after an injection there is a primary hypocalcified layer present in both enamel and dentin. This disturbance is probably brought about by the deposition of calcium fluoride in the tissue rather than the normal calcium salts (PO_4 or CO_3). A secondary effect consisting of a normal or hypercalcified layer is seen immediately following the hypocalcified stripe.[77] (Figs. 79 and 80.)

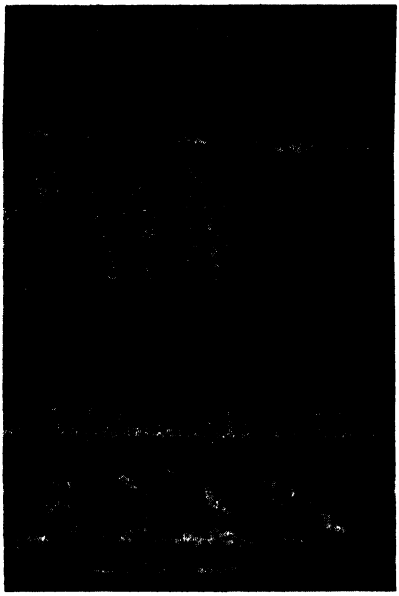


Fig. 86. Photomicrograph of the posterior portion of the enamel epithelium of the upper incisor of a rat given one injection of 0.3 cc. of 2.5% NaF and killed 24 hours later (× 470). Note the globules (Gl.) within the ameloblasts and the irregular surface of the organic enamel matrix. S.I., stratum intermedium; O.E.E., outer enamel epithelium; L.A.P., labial alveolar periosteum. (From Schour and Smith.[75, 76])

A hypocalcified stripe followed by a hypercalcified reaction is not peculiar to fluorosis but can also be seen in other experimental conditions.[57, 78] The combined effect is a calcio-traumatic reaction rather than a specific response.[79] Microchemical analysis may lead to a differentiation of the calcio-traumatic effect in different experimental conditions.

The first *gross* manifestation in the incisor is a lack of pigmentation or a dull chalky opacity in the enamel when the affected portion of the tooth has erupted into the oral cavity (Fig. 87). The recovery reaction shows a normal pigmentation and calcification. Thus, multiple injections of sodium fluoride produce an alternating zebra-like ring pattern of unpigmented, poorly calcified enamel corresponding to the number of injections[75, 76] (Fig. 87).

Growth. The rate of appositional growth is not disturbed when injections of small doses of sodium fluoride are given (0.3 cc. of a 2.5% solution). When the dosage is increased or the injections are given at intervals of 24 hours, so that a cumulative effect is produced, the formation of enamel and dentin is severely disturbed. The higher dosage causes a complete arrest in growth and hypoplastic defects, whereas small doses produce lines of deviated growth.[75, 76, 80]

Chronic Fluorosis. In the early stages, the effects produced by adding NaF to the basal diet are essentially similar to the effects produced by injections. Smith and Lantz[81] noted a loss in pigment two weeks after the rats had been placed on a diet containing .025% sodium fluoride. However, the prolonged cumulative action of the fluorine produces a further toxic effect upon the formative cells. The disturbance in calcification progresses so that the incremental stratification becomes accentuated and in the enamel the matrix tends to persist in an immature, acid-resistant stage. Still later, the appositional growth is disturbed, producing vascular inclusions in the dentin and hypoplastic defects in the enamel which tend to recur rhythmically. Chemical analysis shows that with a concentration of sodium fluoride over 0.1% of the ration the teeth and bones were lower in ash content but showed an increase in calcium percentage and a decrease in phosphorus percentage.[82] When the diet contained .05% of sodium fluoride in the ration, there were no significant alterations upon chemical analysis. Histologic analysis, however, showed the early effects described above and is, therefore, the more delicate and useful method of analysis. The fluorine content of the bones and teeth increases with the proportion of the fluorine added to the diet.[83]

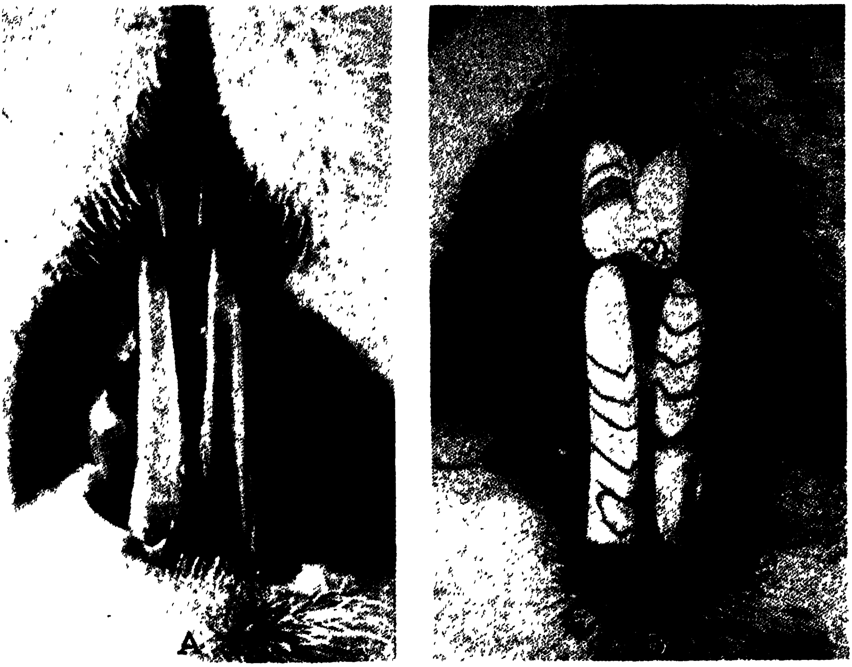


Fig. 87 (A) Frontal view of normal rat incisors showing the typical translucent appearance. The enamel is covered with an orange pigment.

(B) Frontal view of the incisors of a rat which received eight injections of 0.3 cc. of a 2.5% NaF solution at 48-hour intervals. Note the pigment-free, chalky white, hypocalcified areas produced by the injections, and the ringed appearance of the tooth. (From Schour and Smith.[75, 76])

Eruption. The rate of eruption is retarded in late chronic fluorosis,[84] the decrease being proportional to the percentage of fluorine in the diet. Sodium fluoride was also found to retard the rate of body growth and to lower the retention of calcium and phosphorus. Smith[84] suggests a probable relation between these three processes because of the similarity in the degrees of the effects produced by the sodium fluoride (Fig. 83).

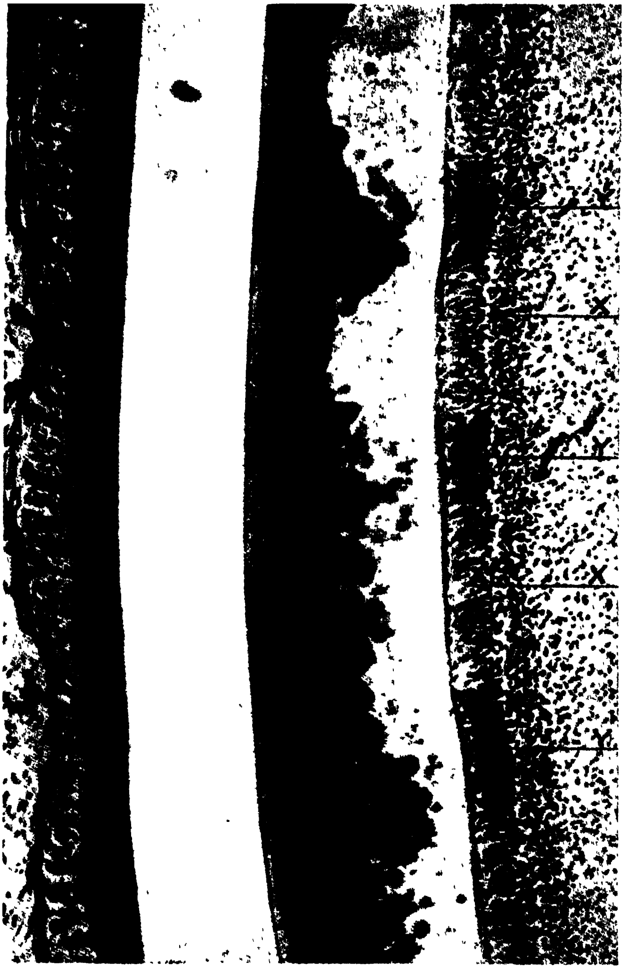


Fig. 88. Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-eight days; *x*, group of atrophic odontoblasts and cessation of calcification; *y*, group of vital odontoblasts and continued calcification. The specimen (1261A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1. (From H. Becks and W. B. Ryder: Experimental Rickets and Calcification of Dentin. Arch. Path., 12:358-386, 1931, Figure 8.)

Fluorosis and Caries. The molar teeth will show mottling when the rat is placed on a fluorine-containing diet at birth or soon after.[85] This is similar to the mottling commonly seen in children living in communities where the water supply contains more than one p.p.m. of fluorine. Miller[86] has found a lowered incidence of caries in fluorine-fed rats. Since the molar teeth are similar to those of man and since children with mottled teeth have been found to be less susceptible to caries than normal, the correlation is at least provocative.[87] Miller[86] suggests that the fluorine acts locally by inhibiting the enzymatic activity of the caries-producing organisms.

Salt-Deficient Diets

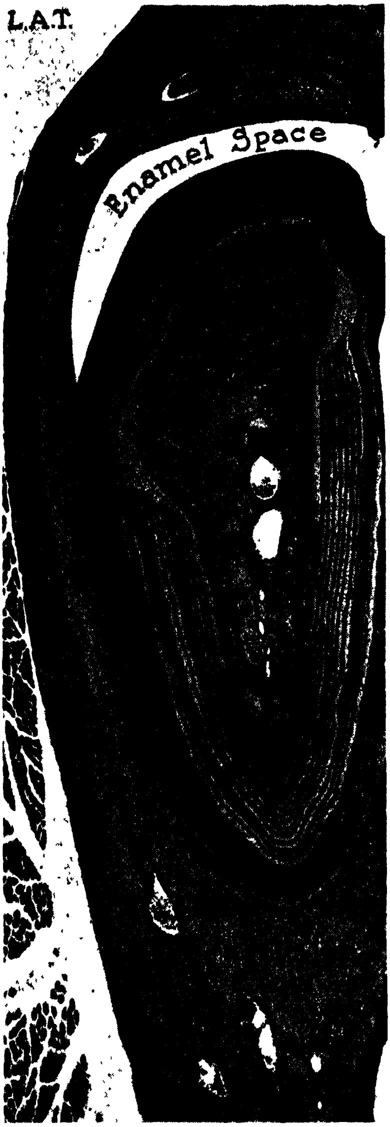
Too often the importance of the inorganic constituents of the diet is overlooked for the more spectacular or interesting investigations in the effects of the vitamins or the endocrines. This field, therefore, offers important possibilities for research. Similarly, the effect of deficiencies in essential amino-acids upon the teeth has apparently not been investigated.

Total Salt Deficiency. When all inorganic salts are omitted from the diet disturbances in calcification are seen in the wide predentin and marked rarefaction of the alveolar bone. Disturbances in formation also occur. Irregular dentin and vascular inclusions are produced. In severe deficiencies the incisors and alveolar bone are mere shells and fracture readily. Formation of secondary dentin in the molars is very irregular. The bone marrow shows an increase of adipose tissue.[88] Clark and Smith[89] found an increased moisture and a reduction in ash content in the incisors of rats fed a diet poor in salts.

Calcium and Phosphorus Deficiency. Gaunt and Irving[56] reduced the calcium and phosphorus content of the diet and found wide predentin and vascular inclusions in proportion to the calcium and phosphorus deprivation in the dentin. Their work and that of others shows that in disturbances in calcium metabolism, the teeth are calcified at the expense of the bone, in accordance with Erdheim's calcio-protective law.[6] The bony skeleton shows marked "decalcification," while the teeth show no resorptions or "decalcifications."

Erdheim's calcio-protective law[6] states that certain tissues are less susceptible to defects in calcium metabolism than are others. The literature contains considerable evidence in the support of this law.[90, 54] Enamel and dentin are not so readily disturbed in their calcification as is the bone; nor are they subject to calcium withdrawal. Thus the bone gives up its calcium readily in case of need, and the enamel and dentin do not. In fact, the calcium and phosphorus made available by the bone are avidly taken up by the calcifying enamel and dentin. When the skeleton is thus deprived of calcium, the growing dentin not only receives the necessary amount of calcium but in addition may receive an excess supply and become hypercalcified (Figs. 94-95). Enamel and dentin thus continue to form and calcify during periods of salt deprivation at the expense of the bone.[89]

Magnesium Deficiency. Rats placed on a magnesium-deficient diet soon show disturbances in the calcification of the teeth. The dentin is markedly interglobular and shows prominent stratification. The enamel epithelium



· Fig. 89. Photomicrograph of a cross-section of a lower incisor of a rat 109 days of age that was placed on a magnesium-deficient diet during the last 41 days of life (× 40). Note the striking stratification in the cementum covered dentin and the reduced width of the enamel covered dentin. Cem., cementum; L.A.P., labial alveolar periosteum; P.D.M., periodontal membrane; Pre., predentin; Str. Z., zone of prominent stratification. (Courtesy of Drs. J. Gagnon and M. C. Patros.)

shows atrophic and hypoplastic changes[91] (Fig. 89), and the dentin is irregular in width. The alveolar bone shows marked atrophy.[92]

Effect of Beryllium Carbonate in the Diet. Glasstone and Robison[93] fed pregnant rats a diet containing beryllium carbonate and found that rickets was produced, probably due to the formation of insoluble beryllium phosphate. The embryos were small, the tooth germs did not differentiate at a normal rate and the formation and calcification of dentin were markedly delayed.

EFFECT OF X-RAYS ON THE TEETH

Leist[94] and Smith[62] found that large doses of x-rays had a selective action in retarding the differentiation of the odontoblast without causing any effect upon the ameloblasts.

THE EFFECTS OF THE ENDOCRINES ON THE TEETH

The endocrines constitute a very important factor in the internal environment of the tooth (with the exception of the mature enamel and dentin which are avascular) as well as of other organs. While the endocrines do not increase the inherent growth potential of the tooth they do serve to regulate its expression.

Hypophysectomy

Eruption. The first effect of hypophysectomy is a retardation in the eruption of the incisors and molars (Fig. 90). In the incisor the rate of eruption is retarded within one week after the operation and thus may be used as an early and reliable indicator of the success or failure of the operation. The eruption is progressively delayed so that a complete cessation can be noted in animals that survive the hypophysectomy one year or longer[17] (Fig. 29).

The changes are not due to injuries incident to the surgical operation, since animals which are unsuccessfully operated upon show a normal eruption and histologic picture.[17] Injections of growth hormone, if given within six to eight months after the operation, increase the rate of eruption in those animals but do not bring it to its normal level (Fig. 90). The normal expression of the eruption potential cannot be attained in the hypophysectomized animal even by replacement therapy.[17]

Growth. Appositional growth at the base of the incisor, although retarded, persists in spite of complete cessation of the eruptive processes and of the forward movement of the apposed tissue. In hypophysectomy we are thus concerned with an interesting dissociation experiment whereby eruption and growth are "dissected." The disproportion between growth and eruption results in the displacement of the odontogenic epithelium in hypoplastic defects, and in a crowding and accordion-like folding of the newly formed and still uncalcified enamel and dentin of the basal end (Fig. 98). In addition, the accumulation of the continuously apposed dentin, which with a normal rate of eruption would have been worn off at the incisal edge, results in the dentinification of the pulp and its gradual obliteration (Fig. 98). Similarly, the cementum may increase from the normal width of 4 μ to 16 μ. By taking weekly roentgenograms of their incisors it is possible to trace the progress and aggravation of the dental changes induced by the removal of the gland.[95]

In the molars the retardation in growth and eruption is seen in the persistence of juvenile characteristics such as short roots and wide apical foramen and the submersion of the teeth (Fig. 91).

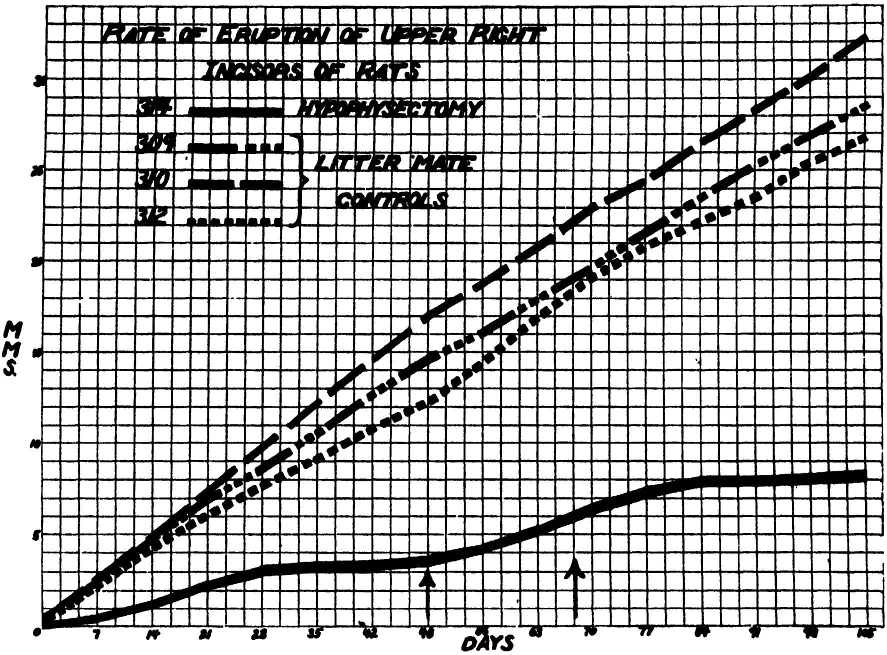


Fig. 90. Graph showing the effect of hypophysectomy and replacement therapy on the rate of eruption of the incisor of the rat. Compare the curve of hypophysectomized rat 314 with those of the littermate controls. Injections of growth hormone were administered for 20 days during the time interval indicated by the arrows. Note the increase of the rate of eruption during the replacement therapy and its eventual decline when the injections were stopped. (From Schour, I.: Recent knowledge of the inter-relationships of endocrines and teeth. Proc. Ninth Inter. Dent. Cong., Vienna, 1:107-128, 1936.)

Calcification. The staining reaction of the dentin indicates a state of overcalcification. In animals of short survival the predentin usually shows many fine isolated calcium globules. It is interesting that this type of predentin (globular predentin) has also been found in adrenalectomy.[96]

Further evidence of an overcalcification is seen in the abnormal sprinkling of hematoxylin-staining globules of various sizes at the cementum surface and the adjacent periodontal membrane of the molars of some of the animals of advanced post-eruptive life.[97]

Periodontal Tissues. The vascular supply to the pulp and periodontal membrane shows a very severe reduction. It is possible that the primary factor disturbing the developmental processes, particularly the eruption, lies in this vascular disturbance.[17, 18] The epithelial attachment is atrophied. The alveolar bone shows a crowding of cementing lines.[95]

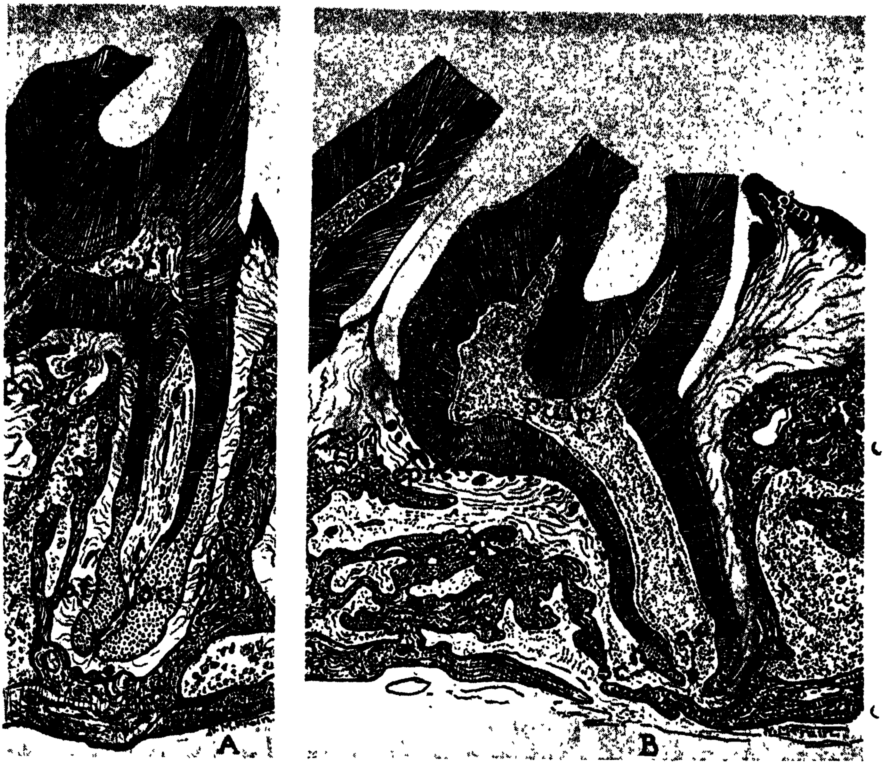


Fig. 91. (A) Semi-diagrammatic reconstruction of the distal portion of the upper first molar of a normal rat.

(B) Similar reconstruction of the third molar of a hypophysectomized rat. The experimental animal died at the age of 309 days, but the histologic picture of the tooth is that of a much younger animal. Note the retarded eruption as indicated by the common level of the gingival crest (gm.) and the occlusal surface of the tooth, the atrophy of the epithelial attachment (ept); the height of the pulpal horns (ph); the reduced blood supply of the gingivae, periodontal membrane and pulp; the increase in the size and number of the epithelial rests (epr); the wide pulp chamber, pulp canal (p), and apical foramen (af); the scant amount of secondary cementum (sc); alveolar bone (alb). Compare with Figure A. sd., secondary dentin which has filled the pulpal horns. (From Schour.[95])

Parathyroidectomy

The parathyroids play a leading role in regulating the blood-calcium level and therefore also the calcification of the teeth. They seem to have little or no direct effect on growth or eruption.

Calcification. The primary effect of parathyroidectomy is a lowering of the blood-calcium level. In the dentin a disturbance in calcification is registered as (a) a hypercalcified effect for the first twenty days after the operation; and (b) a subsequent deficient calcification[26, 98, 79] (Figs. 92-93). This deficiency in calcification is characterized by an irregular and accentuated alternation of zones of different degrees of under and over calcification (Figs. 92, 93, 97, 98). Radiographs of the incisors often showed radioluscent incremental zones which corresponded with the eosin-staining zones in decalcified sections. Fractures of the incisors are common in long survivals. The effects of parathyroidectomy are alleviated by replacement therapy with parathyroid extract. Similar results are obtained when large doses of vitamin D are administered instead of parathyroid extract.[79] This close interrelationship in the action of a hormone and a vitamin may be significant.

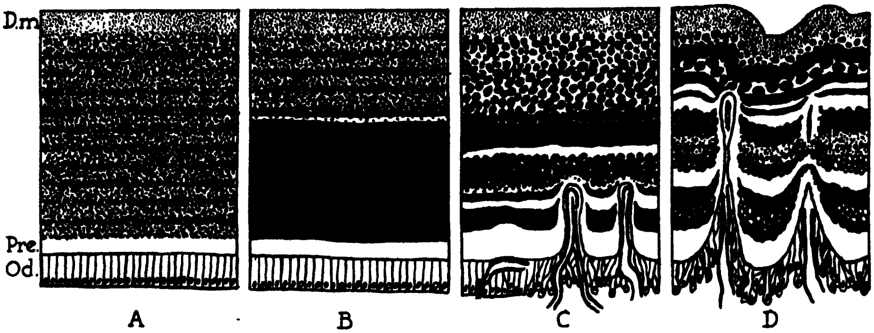


Fig. 92. Schematic representation of the state of calcification of dentin of the incisor of the normal rat (A), and parathyroidectomized rat (B, C, and D).

A Stippling represents normal dentin and shows the daily calcification and growth rhythm The predentin (Pre) next to the normal odontoblasts (Od) is even in width and white. The mantle dentin (D. m) is located next to the enamel and is shown in fine dense stippling

B The stippled zone represents the preoperative dentin, the dark line followed by the narrow dotted zone represents the hematoxylin staining line and eosin zone in the dentin that is being formed and calcified at the time of and shortly after the operation The black zone extending to the predentin is the heavy hematoxylin staining dentin found in the early parathyroprivic condition

C. The irregularly staining stratification zones in the parathyroprivic dentin of long survival are represented by the different size and spacing of globules (black) Note the greater width of the predentin and also the odontoblastic changes associated with the vascular inclusions The dentin immediately adjacent to the inclusions like the predentin is white

D. Long survival with repeated pregnancies and lactations The dentin surfaces bordering the enamel and the pulp are distorted The incremental stratification zones are more irregular and show an arcade-like arrangement near the vascular inclusions Odontoblastic changes are more prominent Compare with Fig 93 (From Schour, Tweedy, and McJunkin [78])

The enamel and dentin of the incisors or molars formed and calcified before the experimental period show no changes. This is the case even when the experimental parathyroprivic condition is aggravated in some series by repeated fastings or by repeated pregnancies and lactations. In spite of the severe drain upon the calcium reservoir of the animal, *no calcium withdrawal occurs from the enamel and dentin.*[79]

Hyperparathyroidism

The primary effect of an injection of parathyroid extract in the normal animal is a rise in the blood-calcium level and a corresponding *hypocalcification* of the dentin. Immediately following is a secondary reaction—a return of the blood calcium to its normal level and a corresponding *hypercalcified* stripe in the dentin[78] (Figs. 94-95). This effect is similar to that seen when excessive doses of vitamin D are administered. These findings indicate an interesting correlation between the histologic reaction of the dentin and the blood-calcium level. The secondary hypercalcified effect is prolonged when multiple injections are administered at overlapping intervals (Figs. 94-95).

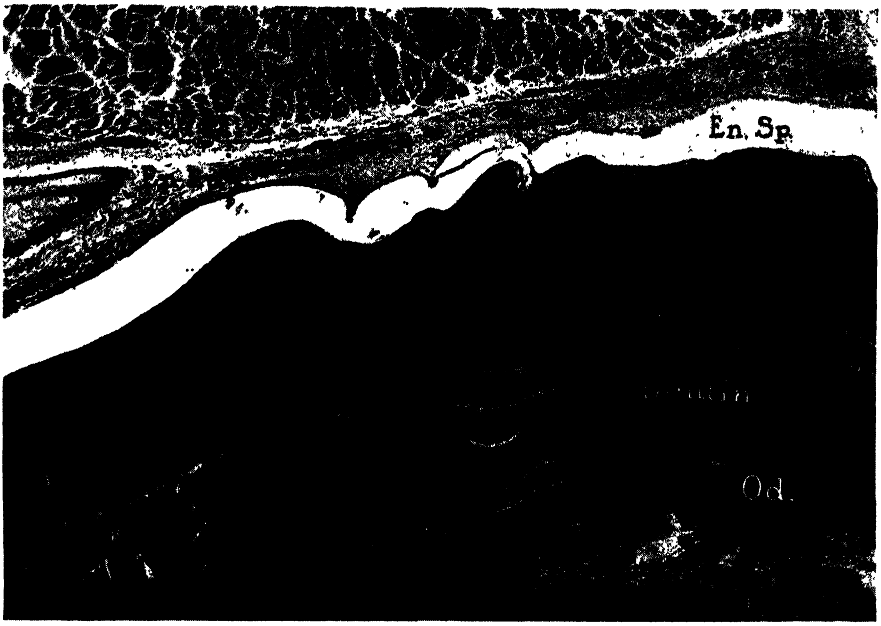


Fig. 93. Photomicrograph of the labial dentin from the distal portion of the upper incisor of a rat which lived 105 days after parathyroidectomy and was subjected to fasting every seventh day (× 23). Note the wave-like disturbance of the dentin-enamel junction and the enamel surface. The parathyroprivic dentin shows the typical irregular calcification zoning. The enamel epithelium (En. ep.) is severely atrophied. L.A.P., labial alveolar periosteum; En. Sp., enamel space; Od., odontoblasts. (From Schour, Tweedy, and McJunkin.[78])

The delicacy of the dentinal reaction suggests the feasibility of using the dentin of the rat incisor as an indicator for a biological assay of the parathyroid hormone in place of the more laborious and expensive method of standardization by means of the blood-calcium reaction in the dog.

In a few animals, hypoplasia was present in the enamel at the level which, according to measurements, was formed at the time of the first injections.

Periodontal Tissues. The alveolar bone showed an abnormal increase in osteoclasts and in addition a fibrous change of the bone marrow, presenting the changes characteristic of osteitis fibrosis[78] (Fig. 95). Changes in the other periodontal tissues were not studied. It should be noted that while the alveolar bone is practically wholly resorbed, the enamel and dentin are not restored; in fact, the dentin forming and calcifying during the experiment appears to be hypercalcified.

In prolonged hyperparathyroidism, Burrows[90] reports a change in the alveolar bone of the rat molars from a spongy to an almost compact structure. This effect is quite different from that seen in acute hyperparathyroidism.

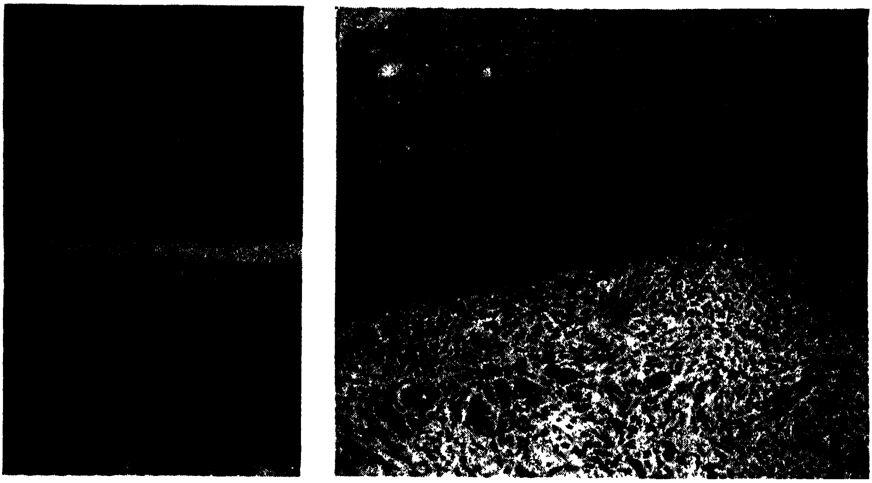


Fig. 94. (A) Photomicrograph of a section of labial dentin of the upper incisor of a rat given four injections of parathyroid hormone and allowed to live 3 days after the last injection. Note the prominent primary hypocalcified (PR) and the secondary hypercalcified (SE) zone in the dentin. D., dentin calcified before the experiment; OD., odontoblasts; P., predentin (× 195). (From Schour, Tweedy, and McJunkin.[78])

(B) Photomicrograph of a section of a molar of a rat 43 days of age that was given 900 units of parathyroid extract during the 4 days preceding its sacrifice (× 130). Note the contrast in response between the bone and dentin. The alveolar bone has become resorbed and replaced by fibrous tissue and osteoclasts (OC). The dentin which was calcifying during the experiment shows hypercalcification (Hy). Od., odontoblasts; P.D.M., periodontal membrane. (Courtesy of Dr. F. C. McLean.)

Hypothyroidism

Thyroidectomy results in a retardation of eruption. The possible correlation between the lowered basal metabolic rate and retardation in eruption offers a fruitful field for dental research. The possible effects upon growth and calcification and upon the periodontal tissues have not yet been sufficiently investigated and present a very promising field of dental research.

Hyperthyroidism

Injections of thyroxin in new-born rats result in a precocious eruption of the incisors on the third day or later, depending upon the dosage.[100] Enamel formation is also accelerated but its structure is normal.[101] Herzberg[1] has found an increased rate of eruption.

Thyroparathyroidectomy

Thyroparathyroidectomy at birth results in a severe retardation of growth and skeletal development. The rates of growth of the dental tissues and the rate of eruption are markedly retarded.[102, 103] The changes are less severe if the operations are performed at later ages. Changes in calcification and in the periodontal tissues were not mentioned.

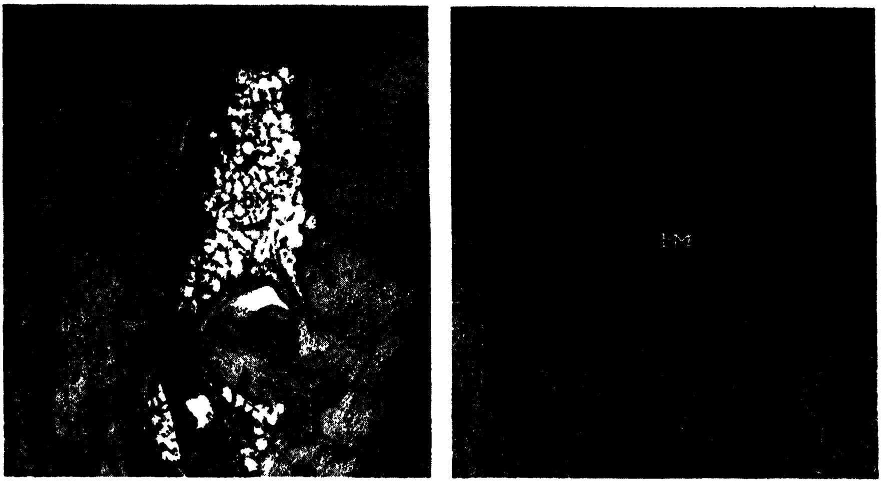


Fig. 95. (A) Photomicrograph of a section of alveolar bone near the lingual alveolar crest of the upper incisor of a normal rat (× 71). Note the rich number of osteoblasts (OB) lining the bone marrow space (BM). P.D.M., periodontal membrane.

(B) Photomicrograph of a corresponding section of a rat which received 14 injections of parathyroid hormone (× 150). Note the absence of osteoblasts; the fibrous change of the bone marrow (FM) and the presence of a number of osteoblasts (OC). Compare with A. (From Schour, Tweedy, and McJunkin.[78])

Parathyroid replacement therapy was not effective in promoting normal growth. Powdered dry thyroid gland did, however, produce a normal growth response.[102] It would appear that the parathyroids are primarily concerned with calcification and that the thyroid is concerned primarily with growth and eruption.

The effects of multiple endocrinectomies may offer clues that are not apparent in the absence of single glands.

Adrenal Insufficiency

The complete removal of the adrenals in the rat produces characteristic disturbances in the calcification of the dentin. The predentin which normally shows no calcospherite formation is sprinkled with hematoxylin-staining globules—globular predentin[96] (Fig. 96). This suggests a ***premature*** calcification of the dentin. In addition, an overcalcification of the post-operative

TABLE XII

Summary of the effects of endocrines upon tooth development

Experimental Condition	Growth	Calcification (of Hard Tissues)	Eruption	Pathognomonic Syndrome
Hypopituitarism	Slightly retarded.	Slightly increased calcification of dentin; sclerotic alveolar bone. Occasional calcification of periodontal fibers.	Marked progressive retardation and final cessation.	Marked retardation in rate of eruption. Vascular supply of pulp and periodontal tissues markedly reduced.
Hypoparathyroidism	No effect except in very advanced stages.	Increased calcification in early survival; irregular calcification in later survival.	No measurable effect.	Irregular calcification.
Hyperparathyroidism	Occasional acute injury to cells.	Acute primary hypocalcification followed by secondary hypercalcification of dentin. No calcium withdrawal from dentin. Calcium withdrawal from bone followed by osteitis fibrosa cystica.	No measurable effect.	Acute primary hypocalcification and secondary hypercalcification of dentin. Cellular disturbances with rarefaction of bone. Concomitant changes in blood calcium.
Hypothyroidism	*	*	Retarded.	*
Hyperthyroidism	*	*	Accelerated.	*
Adrenal Insufficiency	*	Premature and over calcification of dentin.	No measurable effect.	*
Thymus (Excess)				

* Data insufficient The effects of hyperpituitarism, hyperadrenalism, the gonads and the isles of Langerhans upon the teeth merit further investigation.

dentin occurs. In a small number of animals the enamel-forming cells showed minute intracellular globules which stained prominently with hematoxylin. These changes are not found when the animal has accessory adrenal bodies. No significant alterations in growth or eruption could be observed within the necessarily short survival period.

Thymus

Rowntree et al[104] found that rats injected with thymus extract (Hanson) showed general precocious development and precocious eruption following treatment of successive generations. The incisors which normally erupt in the eighth to tenth day were found to be erupted at birth in the eighth generation of thymus-treated rats.[105]

It is interesting that this precocious development stops when the rat has reached its normal adult development. Thus the growth potential is expressed earlier than normal but is not exceeded.

EFFECTS OF MECHANICAL INJURIES ON THE TEETH

Eruption. The effect of mechanical injuries upon the incisor of the rat is primarily upon the eruption process. When the rodent incisor is freed from occlusal stress by fracture or by the cutting off of its exposed portion, its eruption is accelerated to more than double its normal rate until occlusal contact is restored.[106, 15]

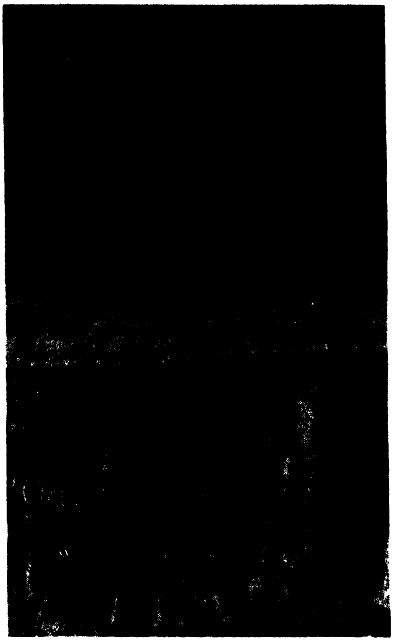
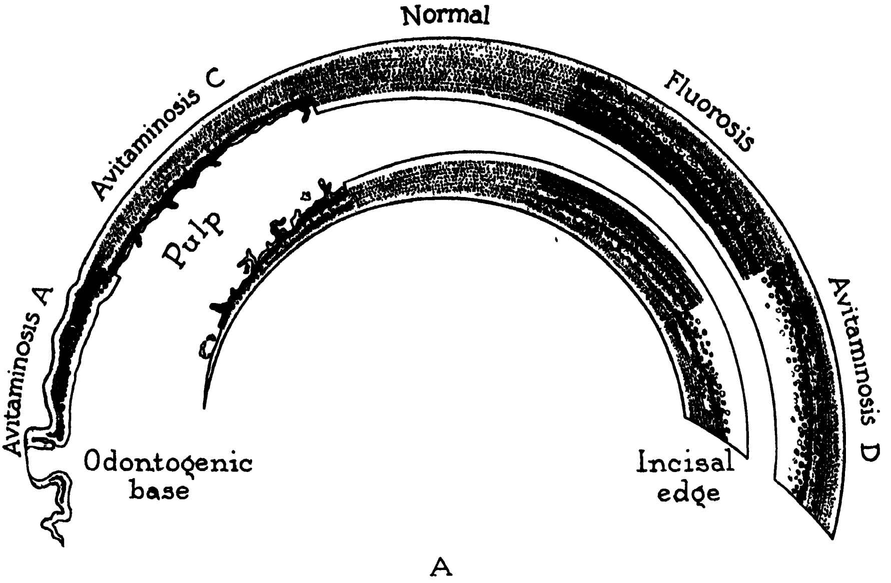


Fig. 96. Photomicrograph of the labial dentin of the upper incisor of a rat that survived nine days following bilateral adrenalectomy. Note the characteristic globular predentin (Gl). Od., odontoblasts (× 420). (From Schour and Rogoff.[96])

On the other hand, traumatic occlusion produced by placing high crowns upon the rodent incisors results in a marked retardation or cessation of eruption. A similar retardation occurs when the proximal fragment of a fractured tooth becomes ankylosed.[15] The roentgenograms of these teeth show an accumulation and folding of calcified tissues at the basal zone and a dentinification of the pulp that is similar but less intense than that seen in the incisors of the hypophysectomized rats. Appositional growth continues in spite of retardation or cessation of eruption. We thus have another instance of an experimental dissociation of growth and eruption.

It appears that the normal rate of eruption represents only a partial fulfillment of the eruption potential. The expression of the eruptive force is frustrated in the presence of excessive occlusal stress, while its full realization is approached in the absence of antagonists. In the latter condition, which



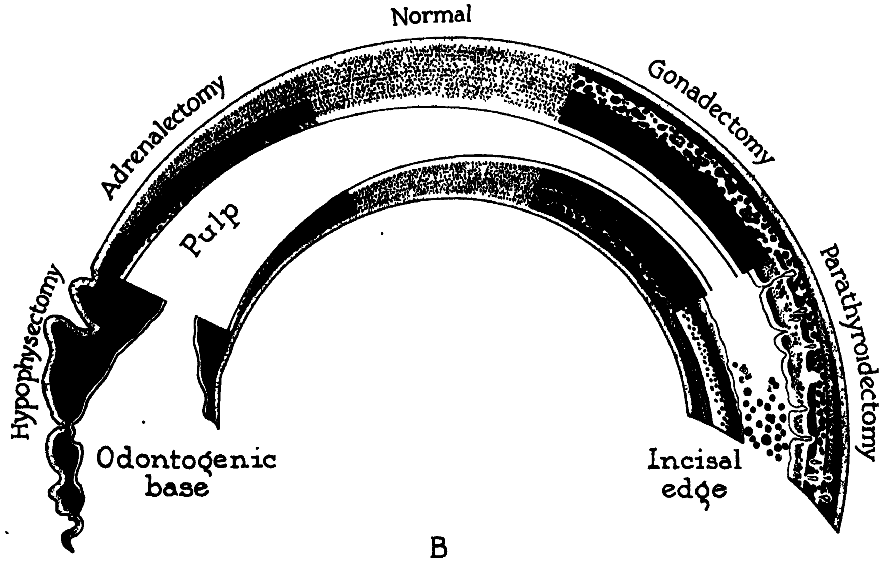


Fig. 97. Composite diagrams indicating the characteristic disturbances in the formation and the calcification of the dentin of the rat incisor associated with (A) particular dietary interferences, and (B) particular endocrine dysfunctions. (The changes in gonadectomy were found only in the ground squirrel.) (From Schour.[9])

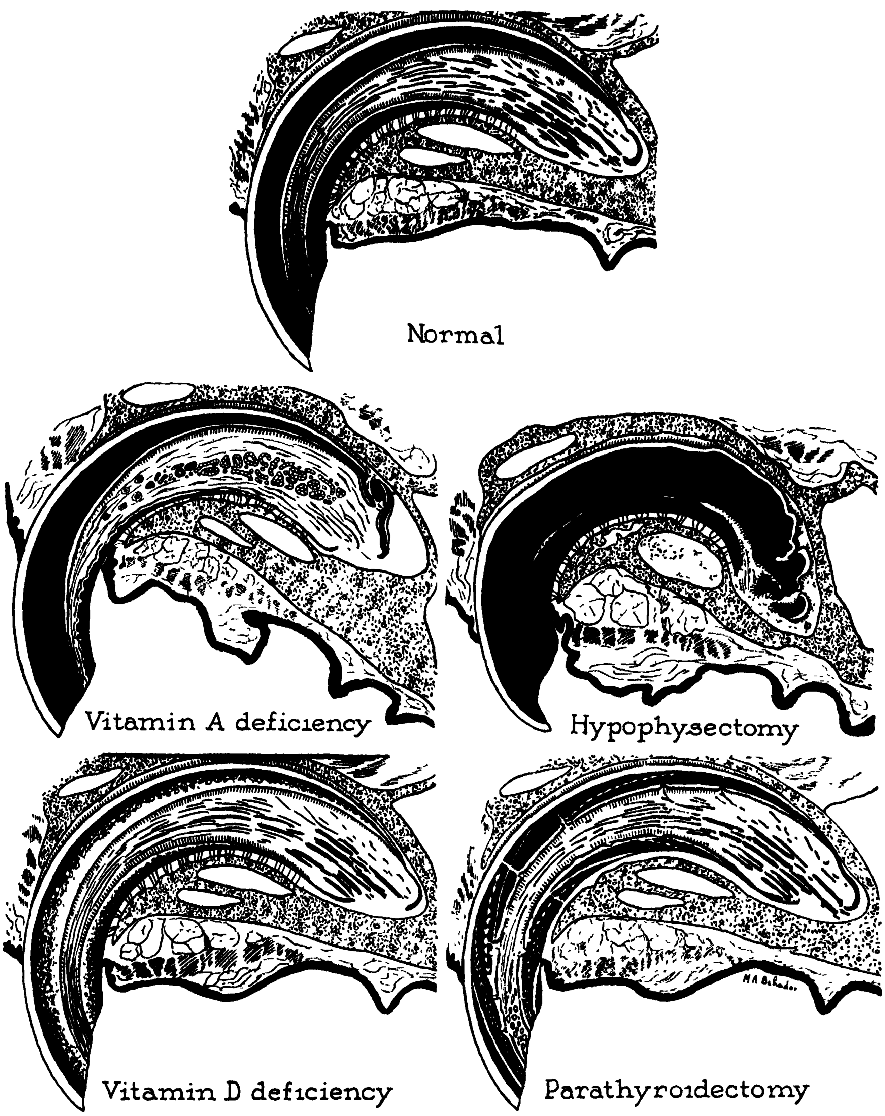


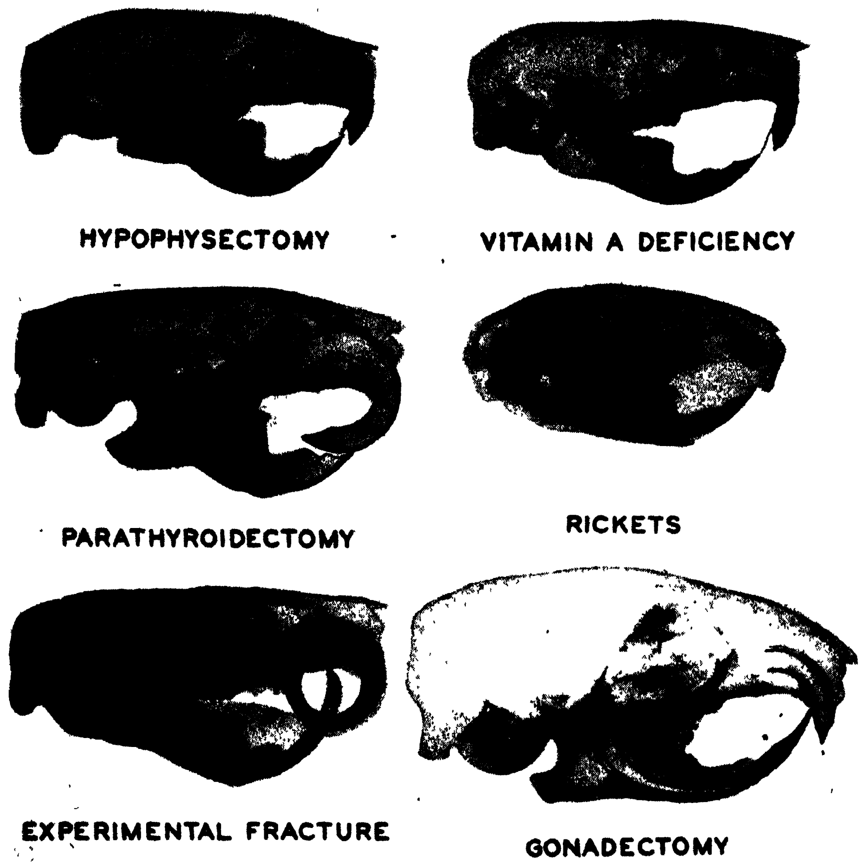
Fig. 98. Semi-diagrammatic tracings of the upper incisors of a normal rat and of rats subjected to specific experimental interferences. The pathognomonic effects that can be recognized in midsagittal histologic sections are shown. Compare with figure 99.

Vitamin A deficiency Note the thicker labial and the thinner lingual dentin resulting from the altered gradient of appositional growth, the islands of epithelium and atypical dentin within the pulp resulting from the disturbed proliferation of the odontogenic epithelium and differentiation of the formative cells

Vitamin-D deficiency. Note the wide predentin and the markedly interglobular dentin zone resulting from the disturbance in calcium metabolism

Hypophysectomy. Note the smaller size, distortion of form and the dentinification of the pulp resulting from the retardation in eruption

Parathyroidectomy. Note the irregularly accentuated alternation of zones of different degrees of calcification resulting from the alteration in calcium metabolism, and the vascular inclusions in the dentin.



HYPOPHYSECTOMY

VITAMIN A DEFICIENCY

PARATHYROIDECTOMY

RICKETS

EXPERIMENTAL FRACTURE

GONADECTOMY

Fig. 99. Radiographs of the heads of rats subjected to various experimental interferences. The calcified portions of the upper and lower incisors are traced in black. The changes seen in the roentgenograms alone are often sufficiently characteristic to make them of diagnostic value. (The changes in gonadectomy are those observed in the ground squirrel.)

Hypophysectomy Note the filling-in of the pulp, the folding of the dentin at the basal end and the distortion of form due to the marked retardation in eruption.

Parathyroidectomy. Note the shell-like appearance of the incisor due to the markedly deficient calcification and formation of the dentin. Note also the elongation of the upper incisor due to the fracture of the lower.

Experimental fracture. Note the spiral elongation of the upper incisor and the perforation into the palate due to the removal of its lower antagonist by fracture.

Vitamin-A deficiency. Note the increased thickness of the labial dentin and the decreased thickness of the lingual.

Vitamin-D deficiency (*Rickets*). Note the shell-like incisors and the fracture of the upper due to deficient calcification of the dentin.

Gonadectomy (*Ground Squirrel*). Note the shell-like incisors and intra-alveolar fracture of the upper due to deficient calcification.

may result from malrelationship of incisal bevels or fractures, the normal attritional wear does not occur and the characteristic spiral "elongation" is produced (Fig. 70).

Effect of Fractures on Distant Teeth. In a carefully controlled series of experiments, fractures of the intra-alveolar portions of one or both lower incisors resulted in hypoplastic defects and retarded eruption of the *remaining* uninjured incisors. This unexpected and surprising reaction is not due to trauma and has been tentatively explained on the basis that during the injury of the formative dental cells at the site of fracture, specific cytolysins are possibly released which have a harmful effect on homologous cells in distant teeth.[15] Considerable further experimentation is necessary before this hypothesis can be validated.

Extraction of Molars. Extraction of the upper right first and second molars in rats is followed by extensive alterations in the dentin, pulp, gingivae, and alveolar bone of the remaining molars.[88] The investigators (Anderson et al.) emphasized the far-reaching effects of changes in masticatory stress and strain.

EXPERIMENTAL PRODUCTION OF DENTAL CARIES IN THE RAT

Caries is a post-eruptive disease of the dental hard tissues, and is therefore not related to the growth and calcification of the tooth. It begins at the surface of the enamel exposed to the oral cavity and proceeds from without inward. It is primarily a bacterial disease and is in no way directly related to any "metabolic" process of the enamel or dentin.

The rat incisor cannot be used for a study of dental caries because its continuous eruption and wear would soon obliterate any carious process that might arise in the exposed portion.

The molars, on the other hand, remain in the oral cavity for a much longer period of time, and in addition present pits and fissures which foster and protect the carious process. They are similar in form and function to the human molars. The minor differences that are present should, however, be kept in mind (flat approximal contacts, enamel-free dentin cusps, liability of cuspal fracture, rodential-unilateral-occlusion, etc.).[25]

Caries has been produced experimentally by the use of a diet which is characterized by the physical size of its *particles* rather than by the presence or absence of any given nutritional elements.[25] The latter, however, probably play some secondary role in the production of caries, since the caries incidence has been raised or lowered to varying degrees by modification of the nutritional factors (carbohydrates, vitamins, etc.). The action of fluorine in inhibiting dental caries is discussed in the section on fluorosis.

SUMMARY AND CONCLUSIONS

The teeth of experimental animals serve as biologic indicators which can be used to assay the effects of experimental interferences. Such assays need not be qualitative only but with the use of the methods described can also be quantitative. For example, the effect of vitamin-A deficiency upon growth can be quantitatively evaluated by vital injections, and such an analysis reflects the effect of vitamin-A deficiency not only upon the tooth but upon the growth of other organs or tissues (bone). There are strong indications that the growth of the long bones and skull can be studied in a similar manner and with equally promising results in the normal and experimental animal. In addition, the effect upon calcium metabolism and calcification and upon eruption can also be measured.

A comprehensive study of experimental effects should include an analysis of the effects on the dentition. Such an analysis will not merely add to our knowledge of tooth development but will often yield data on physiologic processes particularly calcium metabolism and appositional growth, that are not reflected as accurately and delicately in any other organ of the body.

Valuable clues to the solution of physiologic problems are thus waiting to be discovered in the teeth of experimental animals. This laboratory will gladly cooperate in the analysis of such teeth.

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7

The Digestive System

By

THOMAS E. MACHELLA and J. Q. GRIFFITH, JR.

THE DIGESTIVE system of the rat has not been utilized for experimental purposes to the extent that certain established operative procedures suggest that it may be. A description of some of these technics will indicate its potentialities.

METHOD FOR PASSING A STOMACH TUBE

A No. 8 French rubber catheter with a depressed eye is used as the stomach tube. With the rat resting on the table and its head facing toward the operator's right, the left hand of the operator is spread across the animal; the thumb and index finger are so placed that the middle of the distal phalanx of the former is at the angle of the mouth, while the latter straddles the base of the skull of the animal (Fig. 100). This arrangement gives these two fingers control of the head. The palm of the left hand fixes the animal to the table by gentle pressure on its back. In this way all four feet of the animal are immobilized. The thumb is then slid upward and backward, a procedure which draws the tissues back, opens the jaws, and at the same time slightly turns the head of the animal toward the operator (Fig. 100). This position must be maintained until the end of the procedure. The tip of the rubber catheter is moistened with normal saline (not oil) and is inserted over the tongue and passed either along the right or the left side of the mouth, keeping the tube as close to the cheek as possible. By this maneuver the tube moves readily down the esophagus into the stomach (Fig. 101). If any resistance to the passage of the tube is met, the tube should be removed and reintroduced. The length of catheter necessary to intubate the stomach depends upon the size of the animal. With a little experience, impingement of the tip of the tube upon the stomach wall is readily recognized. When measured amounts of fluids are to be injected, the catheter is attached to a Luer syringe, and the fluid is drawn through the catheter before being introduced into the stomach. This permits one to ignore the fluid which remains in the catheter at the end of the injection. After the fluid is slowly injected into the stomach, the catheter, with syringe attached, is quickly withdrawn.

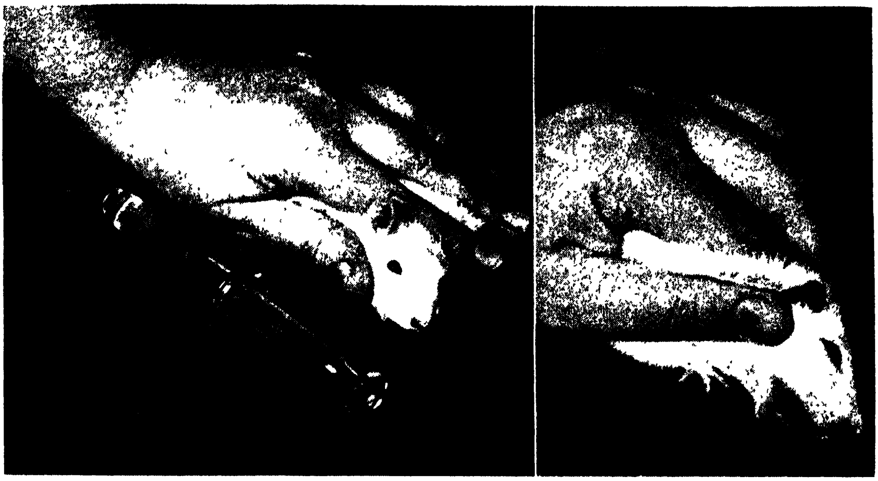


Fig. 100. (*Left*) Method of holding the rat prior to passing a stomach tube. (*Right*) Opening the rat's jaw and turning the animal's head to meet the tube.

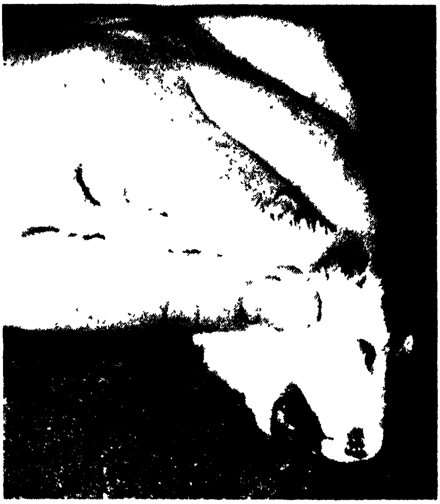


Fig. 101. Inserting the tube into the stomach.

GASTRIC ACIDITY

Using a technic somewhat similar to that reported by Roe and Dwyer,[4] Friedman[5] was able to demonstrate the presence of free acid in the fasting secretion of rats anesthetized with Nembutal or ether. The procedure involved ligation of the duodenum and aspiration of gastric content by means of a No. 18 needle inserted into the stomach in the region of the lumen. Friedman found, in a series of 65 animals, that the average volume of juice secreted during a 30-minute period was 2.0 cc. and that the average free acidity was 70 and the total acidity 80 mEq. per liter. Some of the animals secreted small amounts of acid gastric juice continuously for over 5 hours. The fasting secretion was not inhibited by atropine in doses of from 0.8 to 1.0 mg. per kilo.

Following the subcutaneous administration of histamine phosphate in doses varying from 0.02 to 5.5 mg. per Kg. every 15 minutes no increased secretion of fluid was observed. With the larger doses of histamine (from 3.5 to 5.5 mg. per Kg.), however, the secretion appeared to be more concentrated with respect to acid. Polland[2] found no free acid secreted after huge unstated doses of histamine. On the other hand, Roe and Dwyer[4] believed that a dose of 0.03 mg. per kilogram of histamine phosphate did stimulate a secretion of acid. Their curves for volume and acidity obtained in the histamine-treated rats paralleled closely those obtained by Friedman[5] for his animals that received no histamine.

Komarov and his associates,[6] using a combination of gastric fistula and pyloric ligation in animals under urethane anesthesia, also were able to demonstrate a spontaneous gastric secretion. In their animals the rate was less than 0.1 cc. of gastric juice per hour per 100 grams of body weight. They found that peptic power of the juice ranged between 64 and 400 Mett units and that the chloride content ranged from 150 to 160 mEq. per liter. The introduction of 1 cc. or more of 10 per cent solution of alcohol per 100 Gm. of body weight into the cecum produced a definite but not a large increase in the acidity and volume of secretion. Five per cent alcohol in similar quantities produced equivocal effects. Their results after histamine injection were essentially in agreement with those reported by Friedman. Choline administered subcutaneously in doses of from 7 to 30 mg. per 100 grams of body weight produced a definite increase in the rate of secretion, in acidity and in peptic power.

SURGERY OF THE GASTRO-INTESTINAL TRACT OF THE RAT

The successful performance of various surgical procedures on the gastrointestinal tract of the rat is just as possible as in larger animals providing one is willing to familiarize oneself with modifications necessitated by the small size of the organs. Healing of suture lines will occur quite satisfactorily if the principle of "inversion of peritoneal coats" is adhered to.

Because of the small size of the intestine the use of the smallest sizes of instruments, sutures, and needles greatly facilitates the technic of various operations. The use of small needles for suturing purposes is necessary. Ordinarily, in larger animals a straight needle is easier to handle, but in the rat a small round curved needle, such as a No. 18 M/M Kalts' corneal needle, is preferable. No. 000 silk is sufficiently fine yet strong enough to be satisfactory. The use of small hemostats as intestinal clamps results in irreparable damage to the part of the gut between the blades of the hemostat, hence such hemostats are always removed, after excision of the intervening portion, by transecting the healthy gut near the edge of the hemostat blades, particularly if it is desired to do an "end to end anastomosis." The use of an iris needle holder greatly facilitates the handling of the small needle. It is preferable to use a needle holder which opens by sliding a catch with the tip of the thumb. Finally, whenever wide exposure is desired, the use of a mastoid retractor to separate the edges of the wound is to be recommended.

General Principles Which Apply to Intestinal Suturing in the Rat. 1. Though the ability of the rat's peritoneal cavity to withstand infection is well known, the use of surgical cleanliness is to be recommended. If the intestinal lumen is to be opened great pains must be taken to "pack off" the site of the opening to localize, as much as possible, the effects of fecal spilling. It is best to fold small strips of gauze so that they do not shed bits of thread. Whenever possible, it is advisable to deliver the portion of the gut which is to be opened through the incision and let it rest on the abdominal wall. The abdomen, itself, is covered with a moist saline pack, and the gut covered with other moist packs. The bowel and the packs surrounding it must be kept moist with warm saline.

2. In intestinal suturing the mucosa must be inverted so that serosa is brought into contact with serosa. The interrupted or continuous Lembert stitch accomplishes this. (See Fig. 102.) The esophagus does not have a serosa, hence a different type of suturing is required as will be described under the technic of gastrectomy.

3. The use of fine silk for making anastomoses in the intestinal tract of the rat necessitates the tying of all knots on the outside of the gut, as a silk knot on the mucosal side of a suture line is apt to act as a focus of continuous irritation and result in the production of an ulcer from which sudden massive hemorrhage may occur. The tying of knots on the outside is facilitated if Lembert's principle of coapting peritoneal surfaces is observed.

4. Three guy sutures, such as are used in anastomosing the cut ends of blood vessels in the method of Carrel and Guthrie,[7] will serve to approximate the proper margins of the small lumina of the bowel. By the use of the 3 guy sutures, 3 sides of an equilateral triangle are made, each side being sewn with the long end of the last suture placed.

5. Pre-operative preparation of the rat on which gastro-intestinal surgery is contemplated may include a period of fasting. Satisfactory results are obtained if food is not available during the night preceding the morning of operation since rats eat most of the 24-hour intake during the night.

6. Postoperative withdrawal of food is not necessary except in the case of the gastrectomized animals, as the rats tend to eat very little until the second day after operation. Fluids and glucose should be administered parenterally for at least 48 hours following gastrectomy.

7. Light ether anesthesia is satisfactory for all the surgical procedures to be described.

TECHNIC FOR AN ILEO-ILEOSTOMY

This procedure calls for the excision of a portion of the ileum and for the anastomosis of the cut ends. The technic to be described is intended to serve as a general one for excision and anastomosis of various portions of the gastro-intestinal tract where lumina of equal size are to be anastomosed. Departure from the general technic will be described in the technic for esophago-duodenostomy and ileo-colostomy.

Excision of a Portion of the Ileum. The segment to be excised is delivered through the incision and the surrounding structures "packed off." The course of the blood vessels in the mesentery supplying the loop to be excised is studied and an adequate blood supply to the ends to be anastomosed assured. When it has been decided which vessels to ligate and which vessels to preserve, the mesentery on each side of the blood vessels is slit in a plane perpendicular to the axis of the bowel. This results in a triangle, the base of which is the loop of bowel to be excised, while the apex lies at the root of the mesentery. The blood vessels at the root of the mesentery are ligated and divided between ligatures. Next a fine ligature is passed around each of the

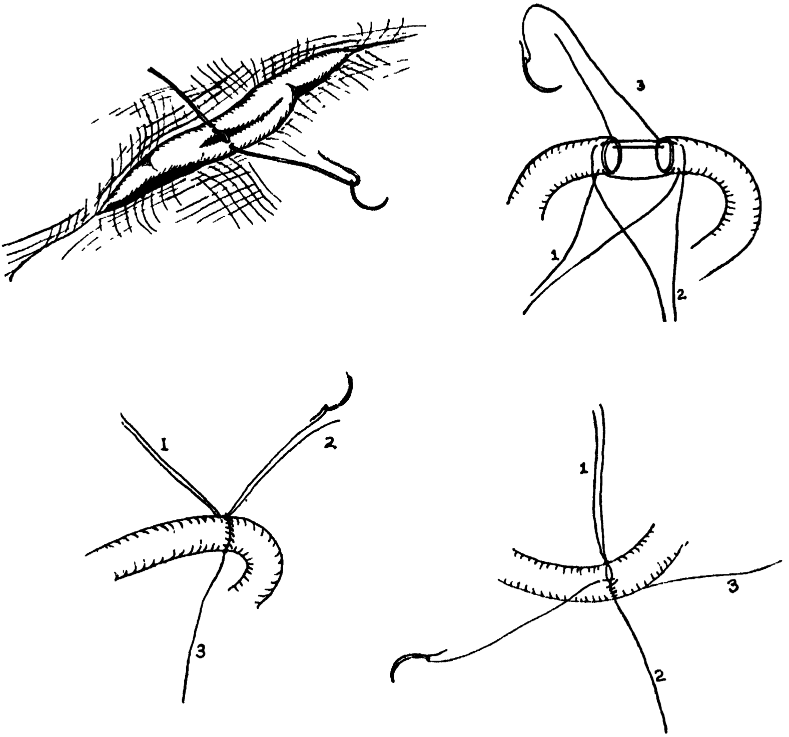


Fig. 102. (*Upper Left*) Interrupted Lembert stitch. (*Upper Right*) Position of 3 guy sutures placed at equidistant points so as to form a triangle. Each suture is placed by Lembert's method so that serosa approximates serosa. The needle remains on guy suture 3. (*Lower Left*) Side of triangle 3-2 sewn by means of continuous Lembert suture. (*Lower Right*) Side of triangle 2-1 in process of being sutured.

primary vascular loops at the base of the triangle and division made between ligatures. The loop to be excised will become cyanotic and excision should include the portion of the bowel beyond the confines of the cyanotic loop. A small hemostat is placed on the bowel just opposite the ligatures on the primary vascular loops and another hemostat is placed inside of the first and as close to it as possible. With a sharp knife the bowel is transected by "shaving" the jaws of the first hemostat placed. The same procedure is repeated on the distal portion of the loop to be excised and now the loop of gut with its attached triangular piece of mesentery is removed by lifting away the two inside hemostats leaving the two external hemostats on the ends to be anastomosed.

End-to-End Anastomosis. In using small hemostats to isolate the loop to be excised, the delicate bowel between the blades of the hemostat has been traumatized and hence is unsuitable for anastomosis. This traumatized segment, with its attached hemostat, is therefore excised by an incision paralleling the hemostat blade. The lumina of the adjacent intestinal segments thus lie open side by side. The main peritoneal cavity must be adequately protected by packing. A No. 000 silk suture about 20 inches long, threaded double for about 6 inches, can be used to perform the entire anastomosis. The lumina of the ends to be anastomosed are opposed with the free and mesenteric portions of the lumina in exact apposition. The first guy suture is laid on the mesenteric border by means of an interrupted Lembert suture. It is not tied as yet but a sufficient length is cut off the main suture to provide a guy about 3 inches long to the ends of which a hemostat is applied. Guy suture No. 2 is next placed in a similar manner in position 2 (see Fig. 102) one-third of the circumference away from guy suture No. 1 on top of the anastomosis. A similar length is cut off the main suture, the ends of which are clamped with a hemostat. Guy suture No. 3 is placed opposite guy suture No. 1 and one-third of the circumference away from guy suture No. 2. The needle is allowed to remain on the long end of guy suture No. 3. This will be used to sew each side of the equilateral triangle thus formed. (Fig. 102.) The three guy sutures are now tied in the order they were placed. Since the Lembert stitch was used in placing the guy sutures, serosa is approximated to serosa at the 3 apices of the triangle and the cut edges of the 3 sides of the triangle tend to invert, facilitating the laying down of the continuous Lembert suture to follow.

The services of an assistant are required to hold the sutures in such a position that the guy sutures actually make a triangle of the bowel lumina. Thus one may avoid sewing the opposite walls of the lumen together and producing obstruction. Guy suture No. 1 is held in the left hand of the assistant, while suture No. 2 is held in his right. He makes gentle but firm tension on the two guys. A heavy hemostat is placed on the short end of suture No. 3 and tension applied to it. In his left hand the operator holds a pair of small straight forceps which are used to invert the cut edge of the gut (avoid trauma by sparing use of forceps!), and in his right, he grasps the holder that carries the needle on which is threaded the long end of suture No. 3. The side of the triangle between guy sutures 3 and 2 is first sewn by means of a continuous Lembert suture. When guy suture No. 2 is reached a knot is tied between one of its ends and the sewing suture, and the short end is cut close to the knot. (Fig. 102.) Next a heavy hemostat is placed on the remaining end of suture No. 2; but with suture No. 1 still remaining in the assistant's right hand, while his left holds the short end of guy suture No. 3, thereby keeping the walls of the triangle separated. The operator then sews the side of the triangle between sutures 2 and 1; one of the ends of suture No. 1, when it is reached, being used to tie a knot. (Fig. 102.) The remaining end of suture No. 1 is cut off. At this stage two sides of the triangle, 3-2 and 2-1, have been sewn and there remains the side 1-3 which is dependent. To bring it uppermost the whole anastomosis is rotated through an arc of 240° by passing the remaining end of guy suture No. 1 and the suture with the needle on it under the anastomosis. Thus apex 3 will take the place of apex 1. The assistant holds the free end of suture No. 3 in his right hand and the remaining end of suture No. 2 in his left, while a hemostat is placed on the remaining end of suture No. 1. Thus again the triangle is made. The operator sews the remaining side of the triangle (1-3), a knot is tied between the short end of suture No. 3 and its long end on which the needle is threaded. Then all sutures are cut short to the knot, the anastomosed bowel is restored to its original position, the first row of sutures having been completed. Next a row of interrupted Lembert sutures is placed to reinforce the first, and the anastomosis is complete. A single suture may be used to unite the edges of the mesentery from which the triangular segment was removed.

GASTRECTOMY

Excision of Stomach. A long, high, midline incision is made and wide exposure is obtained by use of a mastoid retractor. By means of a small hemostat or Allis clamp on the fundus the stomach is pulled toward the midline and down. Next, the relatively avascular membranes between the greater curvature of the stomach and the splenic mesentery and between the stomach and the lobes of the liver are severed. The blood vessels, 4 separate sets, are doubly ligated and cut between ligatures. They must be ligated as close to the stomach as possible in order to spare the branches of these blood vessels which supply viscera other than the stomach. The order in which ligation and section are made are as follows: (1) gastric branches of the gastro-duodenal at the greater curvature; (2) the right gastric or pyloric artery to the lesser curvature; (3) gastric branches of the splenic behind the stomach; and (4) the left gastric artery which supplies the anterior and posterior surfaces of the cardiac end of the stomach. Great care must be taken to spare the branches supplying the pancreas, duodenum, spleen, and lower end of the esophagus. It is better to use interrupted ligatures rather than to attempt to tie off too much tissue in a single ligation and thereby run the risk of damaging a branch to organs other than the stomach. After the various vessels have been ligated a fine pointed hemostat is clamped over the esophagus just cephalad to the junction of the esophagus and stomach. (Care must be exercised not to damage a tiny branch of the left gastric artery supplying the lower end of the esophagus.) An incision is made through two-thirds of the width of the esophagus by means of a knife blade cutting close to the end of the hemostat. A silk suture about 6 inches long on a needle is passed through the uncut one-third in order to act as a traction suture and to prevent slipping of the mucosa of the esophagus up into the lumen of its muscular wall, otherwise this might occur after transection of the esophagus has been completed. (Fig. 103A.) The esophagus is allowed to retract under the diaphragm, whence it can be pulled down at will by the attached suture. Now the fundus of the stomach is raised out of the incision. The stomach is now free except for its attachment to the duodenum. The blades of a small hemostat are applied to the duodenal side of the pylorus and another hemostat is applied just distal to the first. Transection of the duodenum is performed by cutting between the hemostats with a sharp knife. The entire stomach can now be removed from the operative site and preparations made for anastomosis of the esophagus to the duodenum.

Anastomosis of the Esophagus to the Duodenum. The anastomosis can be performed either by the "end to end" or the "side to side" methods. Either may prove satisfactory from an anatomical as well as from a functional standpoint. In both, the aim is to preserve an adequate lumen of the anastomosis, the accomplishment of which is facilitated by the use of the principle of the "3 guy suture" technic of blood vessel anastomosis described previously. Before deciding to make an "end to end" or an "end to side" anastomosis between the esophagus and duodenum, one should first draw up the portion of the duodenum to be anastomosed to the esophageal stump in order to test the end of the duodenum for mobility. If tension is required to bring the duodenum immediately beneath the diaphragm, the operator should elect to perform an "end to side" esophago-jejunostomy. Undue tension on the site of the anastomosis tends to create a pulling apart of the suture line with leakage and subsequent peritonitis or local abscess formation.

End-to-End Anastomosis. The technical feat to be accomplished, if one contemplates this method of anastomosis, is anastomosis of the end of the esophagus which has a small lumen to the end of the duodenum which has a much larger lumen and still preserve an adequate blood supply to the anastomotic end of the duodenum. A method which has been used satisfactorily for accomplishing this aim involves partially closing the lumen of the end of the duodenum. This is performed as follows: The hemostat on the end of the duodenum, which should always be placed with its tip on the free border of the bowel, is allowed to remain in place. With a continuous suture, about one-third of the collapsed portion of the duodenum between the blades of the hemostat is sewn, by undersewing the hemostat tip by means of a through and through stitch. The hemostat is removed, unclamped and reapplied so that the tip of the hemostat now just touches the medial edge of the suture line. The edges of the end of the sutured portion of the duodenum are inverted by means of interrupted Lembert sutures. (See Fig. 103B.) The hemostat is next removed, the portion of the walls crushed between the blades are cut off and preparations made for anastomosis of the cut end of the esophagus to the narrowed cut end of the duodenum, the blood supply to which has been preserved.

Three guy sutures are next placed in position so that they form the apices of an equilateral triangle and the procedure now is the same as that described under the technic of an ileo-ileostomy with one exception. This exception is that instead of placing the guy sutures and the continuous suture of the side of the triangle by the Lembert procedure, as in approximating serosa to serosa, here the needle first passes through the outer wall of the esophagus, through the esophageal mucosa, then through the mucosa of the duodenum and finally out on the serosal side of the duodenal end. It is rather important to catch the mucosa of the esophagus, which tends to retract, in every bite of the needle into the esophagus. After the three sides of the triangle have been sutured, a reinforcing row of interrupted Lembert sutures is

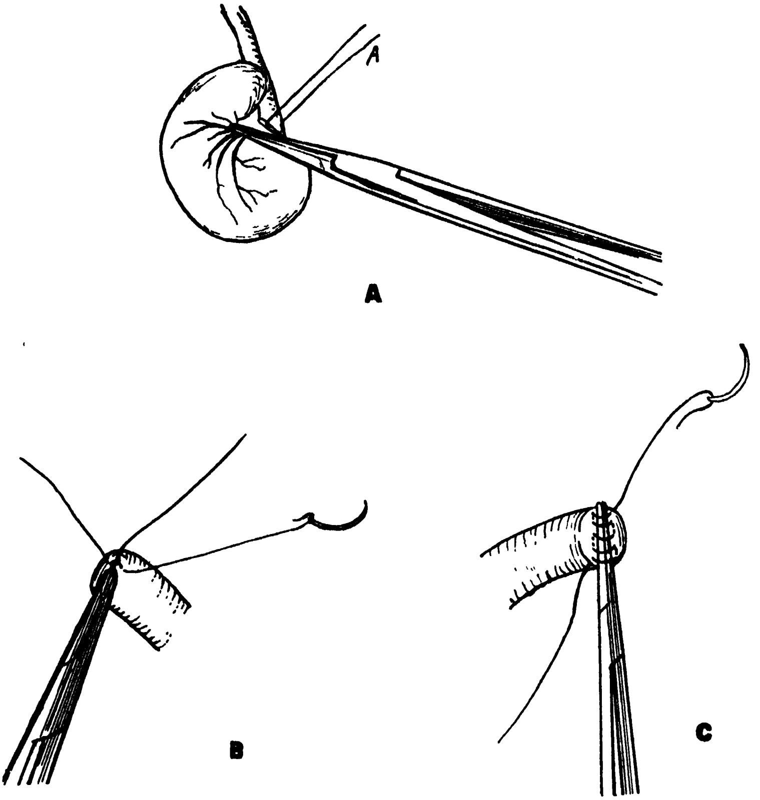
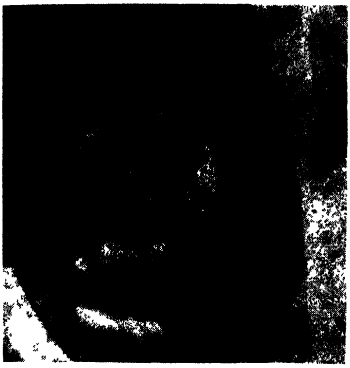
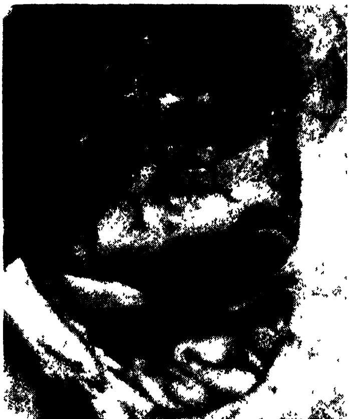


Fig. 103. A. Suture A is placed in the esophagus before the latter is completely transected. The suture is used for traction and should include mucosa to prevent its slipping into muscular wall of esophagus. B. Inversion of stump of duodenum by interrupted Lembert sutures. C. Closure of stump of duodenum by Parker-Kerr method of over-sewing tip of hemostat.

placed. Great caution must be exercised not to constrict the lumen of the anastomosis. After the anastomosis has been completed, it is allowed to retract up under the diaphragm.





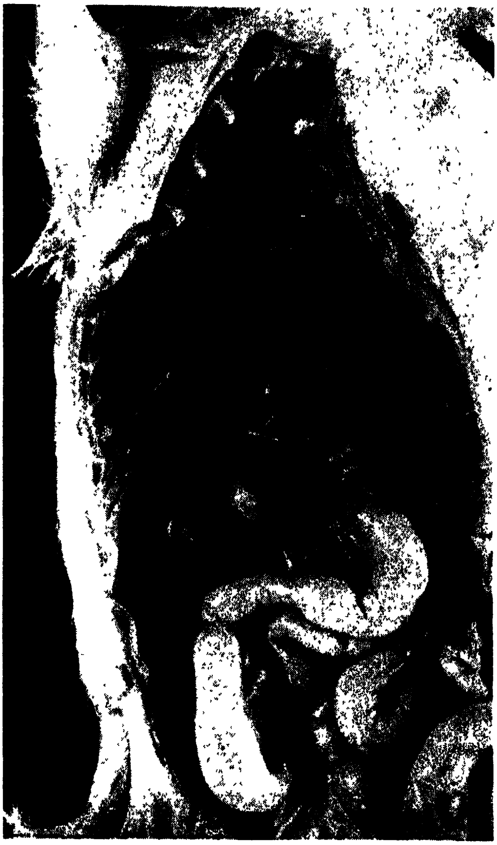


Fig. 104. (*Upper Left*) Photograph of duodenal stump closed by Parker-Kerr method of over-sewing tip of hemostat. Continuous Lembert suture has reinforced first suture placed.

Fig. 105. (*Lower Left*) End-to-side esophago-duodenostomy: first row of interrupted sutures has been placed after closure of duodenal stump.

Fig. 106. (*Right*) End-to-side esophago-duodenostomy one year after removal of stomach.

End-to-Side Esophago-Duodenostomy. If the operator wishes to perform an anastomosis of the end of the esophagus to the lateral wall of the duodenum, the stump of the duodenum is closed over by the Parker-Kerr[8] method of over-sewing the tip of the hemostat, which inverts the cut edges and approximates serosa to serosa. (See Fig. 103C.) A row of interrupted Lembert sutures is then laid down to reinforce the first row. (Fig. 104.)

Next a tiny hole is made in the free border of the duodenum below the end of the stump. This hole is enlarged by inserting the prongs of a pair of forceps into it, until the opening of the duodenum is just slightly larger than the lumen of the cut end of the esophagus. The anastomosis is then completed as in the performance of the end-to-end esophago-duodenostomy. (See Fig. 105.) The anastomosis should not be made too low in the duodenum, as a long duodenal stump may act as a pocket for food.

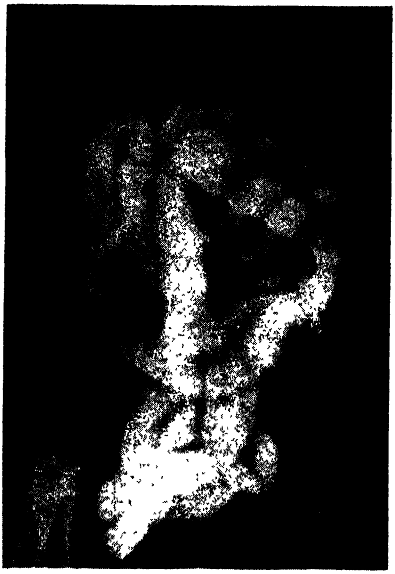


Fig. 107. Roentgen appearance of intestine in gastrectomized rat 3 months after operation.

End-to-Side Esophago-Jejunostomy. If the portion of the duodenum to be anastomosed to the esophagus is not sufficiently mobile to be brought up under the diaphragm and remain there without undue tension, the operator should perform an esophago-jejunostomy. This procedure permits of less tension on the anastomosis and is accompanied by an almost negligible immediate mortality. The technic for the performance of the "end-to-side" esophago-jejunostomy is similar to that for "end-to-side" esophago-duodenostomy.

CECECTOMY

Excision. Similar procedure for excision of the cecum is used as was described above for excision of portion of the ileum.

Anastomosis. The type of anastomosis to be used in anastomosing the cut end of the ileum to the cut end of the ascending colon is an "end to end" but is performed in a manner somewhat different from that described above for the performance of an ileo-ileostomy. This modification is necessitated because of the unequal diameters of the lumina of the ileum and the colon, the latter being larger. The lumen of the ascending colon must be reduced in size sufficiently to correspond to the size of the cut end of the terminal ileum.

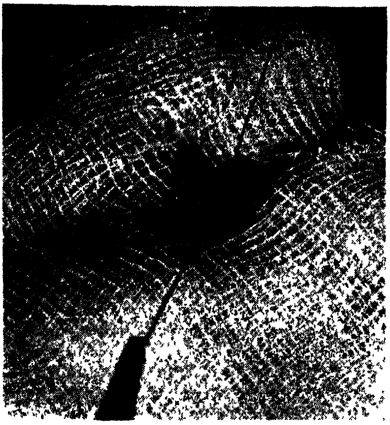


Fig. 108. Stump of colon reduced in calibre and temporarily closed by Parker-Kerr method prior to end-to-end anastomosis to ileum.

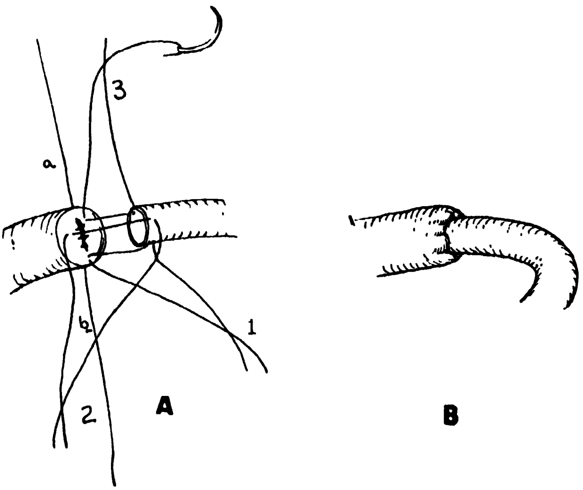


Fig. 109. A. Position of guy sutures in anastomosing end of ileum to temporarily closed end of colon after removal of cecum. Patency of anastomosis is accomplished by pulling on suture a after cutting suture b. B. Completed end-to-end ileo-colostomy.

The end of the hemostat on the cut end of the colon is turned so that the cut edges are turned up. A temporary closure of this end is to be made by the Parker-Kerr method which is performed as follows in this particular case: The first bite of the stitch is made parallel to the bowel axis on the free border of the clamped bowel. The short end of the suture is not tied but attached to a hemostat which is held by the assistant. The stitch now is continued toward the mesenteric border, each stitch being passed across the bowel axis with its loop passing over the hemostat. The last bite of the stitch is made parallel to the bowel axis, the end is cut so that it is about 3 inches long, after which it is held in the left hand of the assistant. The hemostat is unfastened and as its end is pulled through the loops of the continuous suture, the assistant pulls on both of the suture ends. (Fig. 108.) Thus the cut edges are inverted and the pull by the assistant is maintained until the end of the stump conforms to the size of the ileum; when this stage has been reached the two suture ends are allowed to remain attached to hemostats and the open end of the ileum is approximated to the closed end of the colon by means of 3 guy sutures as described under the technic for end-to-end anastomosis of the ileum. (Fig. 109A.) After the first row of sutures has been placed the suture temporarily closing the stump of the colon is withdrawn by pulling on one end and thus the continuity of the lumen of the anastomosis is established. All suture ends are cut and the anastomosis is ready for the reinforcing row of interrupted Lembert sutures. (Fig. 109B.) The gap in the mesentery is closed by one or two interrupted sutures and the abdomen is closed.

GASTRIC LESIONS

Gastric lesions have been produced by various methods. Dodds, Nobel and Smith[9] produced a hemorrhagic gastritis by the injection of posterior pituitary extract. Many workers have reported ulcers and ulceropapillomata in the stomach of rats. A list of causes actually stated or implied by various authors for the development of gastric lesions in rats was compiled by Shay et al.[10] It includes the following: infectious;[11] vitamin A deficiency;[12, 13, 14, 15, 16] vitamin A deficiency plus unknown factors;[17, 18] deficiency of some member or members of the vitamin B complex;[19, 20, 21, 22, 23] malnutrition or unknown deficiency;[25, 26] deficiency of protein or essential amino acids;[27, 28, 29]dietary deficiency plus mechanical irritation;[30] mechanical irritation;[31] nonspecific metabolic factor;[32] excess of fatty acids and lipoids;[33, 34] calcium deficiency;[35, 36] acid-pepsin erosion.[37, 38, 39, 40] Shay et al.[10] have observed ulceration in the stomach following ligation of the pylorus, a procedure which has been used[4, 5, 6] for studying gastric secretion.

MISCELLANEOUS

Brown[41] noted ulcerative lesions of the esophagus in animals fed a long-continued rice diet. The lesions largely healed when synthetic vitamins were administered. Jung and Jones[42] removed the stomach of rats and connected the esophagus with the duodenum by a cannula of magnesium, of the type used in blood-vessel surgery. Animals survived as long as 320 days.[43] Alt,[44] using a similar cannula, performed extensive resection of the small intestine. Harrington, Greaves and Schmidt[45] produced a biliary fistula in rats by three methods: (1) anastomosing the common bile duct and the lower colon by a small silver cannula. The bile thus passes out with the feces. (2) Using a similar cannula, the common bile duct is connected with a glass bulb inserted subcutaneously in the abdominal wall. The bulb has an external orifice and the bile drains externally. This is in effect a cholecystostomy performed through an artificial gallbladder in an animal which by nature has no gallbladder. (3) Using a similar cannula the common bile duct is connected with the vas deferens and the corresponding testicle removed. The bile then drains out with the urine.

Bergeim[46] devised a method for studying the absorption of ingested food in the rat by a simple technic. The food is mixed with a known amount of iron oxide, which presumably is not absorbed. By testing for iron any given stock sample and by investigating the substance and comparing their relative proportions in the stool to that in the diet, the percentage of absorption of the food material can be estimated.

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8

Metabolism

By

C. JELLEFF CARR and JOHN C. KRANTZ, JR.

CARBOHYDRATE METABOLISM

Liver Glycogen. The determination of the fasting liver-glycogen content of the rat is extensively employed in carbohydrate metabolism studies. Pflüger's[1] early method, which consists essentially of tissue solution in concentrated alkali and subsequent precipitation of the glycogen in the cold by the addition of alcohol, is still the basis for most of the present-day procedures. The period of fasting prior to the killing of the animal has been given careful study. In the comprehensive work of Cori and Cori[2] a period of 48 hours was employed. Barbour[3] found a mean of 0.16% of liver glycogen in rats fasted 24 hours and 0.32% for animals fasted for 48 hours. This increased glycogen content of the liver after a 48-hour fast was found also by Cori.[4] Cori's value for the 24-hour fasted rat was 0.10% and for the 48-hour fasted animal 0.397%. In this laboratory a 48-hour fasting period has been considered a standard procedure for rat liver-glycogen determinations. Reindollar[5] working here and employing decapitation as the terminal procedure with male animals weighing between 102 and 163 grams found the average glycogen content to be 0.14% for 30 rats with ± 0.02% as the P.E. of the mean. On 26 female animals Deuel[6] reported a mean liver-glycogen value of 0.10%. The average body weight of these animals was 162 grams and the animals were killed with sodium amytal. It should be noted, however, that Deuel observed a sexual difference in the liver-glycogen content of certain of his experimental animals.

In most instances prior to liver-glycogen determinations on the rat, in this laboratory instead of using the fasting animals the authors[7, 8, 9, 10, 11] have used a basal fat diet. For this purpose cacao butter has been found to be especially suitable. The substance is solid at room temperature and requires, therefore, no container. Its odor is attractive to animals who, as a rule, partake of it liberally. The availability of cacao butter as a food has been studied by Gardner and Fox.[12] Using this fat diet the animals are fasted for 48 hours then fed the cacao butter for a period of 72 hours. Sixty-one male animals, employing this procedure, gave an average liver-glycogen value of 0.15%. When the feeding period was extended to 80 hours, using 30 animals, a value of 0.14% for liver glycogen was obtained. One would conclude, therefore, that within the limits of the experimental error of the liver-glycogen determination, the value for the fat-fed animal under these conditions and that of the 48-hour fasted animal are identical.

A considerable amount of study has been given to the experimental diet prior to the time of fasting. The authors used the following standard diet consisting of:

Whole wheat......	64.5 Gm.	Sodium chloride...	1.0 Gm.
Casein............	15.0 Gm.	Calcium chloride...	1.5 Gm.
Klim.............	10 o Gm.	Fat...............	8.0 Gm.

"Purina Chow" has also been used as the sole source of food during the prefasting period. The diets appear to be interchangeable as far as influencing the liver-glycogen content is concerned. Stein et al.[13] has shown that rats fed on a high-fat, low-carbohydrate diet retain more glycogen in their livers during the 15 to 48 hours fasting period than do rats previously fed a high-carbohydrate low-fat diet.

There is a considerable amount of evidence extant supporting a diurnal variation in the glycogen content of the rat's liver.[14] The consensus and the conclusions of the authors after reviewing the data set forth are that these variations are the results of diurnal differences in food intake and we question whether these variations can be established in the fasting animal.

It has been suspected for many years that terminal procedures employed prior to the determination of liver glycogen exerted a marked effect upon the glycogen content found in the liver upon analysis. Cori[15] states, "It has been shown repeatedly that killing an animal always causes a considerable loss of muscle glycogen and thereby often leads to erroneous results." Cannon[16] attributes this loss to glycogenolysis caused by a hypersecretion of epinephrine, produced under the influence of strong emotion. In view of these assertions it would seem desirable to minimize the intensity of the emotions of the animal by extirpating the liver after anesthetizing, rather than to employ decapitation, exsanguination or some other violent terminal method. The effect of various anesthetics upon the glycogen content of the liver and skeletal muscle has been frequently investigated. Murphy and Young[17] have shown that Amytal, chloralose, chloroform, Dial, numal, and Pernoston all lowered liver glycogen, the first named having the least effect. The method, however, involved frequent sampling which procedure, they suggest, may have adversely influenced their results. Major and Bollmann[18] and Cori[19] concur in the observations on the effect of Amytal; in addition, the former investigators have shown that ether anesthesia causes a marked glycogen diminution.

Reindollar[5] working in this laboratory made an extensive survey of this problem. He compared the glycogen content of the liver after decapitation with the results obtained after the use of barbiturate anesthesia. For the barbiturate studies evipal (N-methyl 5,5′-methylcyclohexenylmalonyl urea) was used as a barbiturate with a substituent group on one of the nitrogen atoms of the pyrimidine nucleus. The other compound, phanodorn (N-desmethyl 5,5′-ethylhexenylmalonyl urea) is devoid of the methyl group attached to the nitrogen atom and is similar to evipal in structure. Male rats weighing between 100 and 150 grams which were previously fasted for 48 hours were used. Five-tenths to 0.8 cc. of a 10% solution of the sodium salts of the respective barbiturates was injected subcutaneously.

The precision of the subsequent analytical procedure employed by Reindollar is evinced by a series of test runs made on fresh beef liver and pooled rat's liver. The results are recorded in Table XIII.

TABLE XIII

Liver glycogen determinations

Material	Number of Determinations	Minimum %	Maximum %	Mean %
Fresh beef liver	6	0.97	1.09	1.06
Pooled Rat's Livers	5	0.03	0.04	0.03

Table XIV combines the results on fasted animals killed by decapitation, anesthetized by sodium evipal and sodium phanodorn respectively. Statistically the values obtained in the three series of experiments show no significant difference. It appears therefore that anesthetizing with evipal or phanodorn is as satisfactory as decapitation as a terminal procedure and certainly eminently more convenient. The authors in unpublished data have confirmed Reindollar's findings with Amytal and although conclusive evidence is not available, it would appear that many of the other barbiturates might serve satisfactorily.

TABLE XIV

Effect of various methods of termination on glycogen

Terminal Method	Animals No	Glycogen Per Cent			Probable Error of single Determination	Probable Error of Mean
		Min	Max	Mean		
Decapitation	30	0.03	0.44	0.14	±0.07	±0.02
Sodium Evipal	32	0.04	0.60	0.23	±0.10	±0.03
Sodium Phanodorn	35	0.03	0.45	0.16	±0.08	±0.02

Weighing the extirpated liver is a practical feature of the determination which deserves some comment. Rapid manipulation is desirable to avoid autolysis of the glycogen which begins immediately with the death of the animal. The authors have constructed supports for centrifuge tubes which contain the strong potassium hydroxide solution. The tube in its support is weighed to the second decimal place and the liver introduced and the difference in weight recorded to the second decimal place. This degree of accuracy of weighing is deemed sufficiently refined for the purpose intended and conforms well to the nicety of the remaining analytical procedure.

Good's[20] modification of Pflüger's[1] method is a great advance in quickness and accuracy and since its introduction the authors have used it exclusively with much satisfaction. The use of the centrifuge for complete sedimentation and the carrying out of the entire procedure in the centrifuge tube have substantially augmented the precision of the original Pflüger method.

The conversion of glycogen to glucose by various hydrolytic procedures has been the subject of much investigation. There seems to be no advantage accruing from the use of one mineral acid in preference to another. Good suggests the use of either hydrochloric or sulfuric acid in 0.6 normal concentration. In this laboratory we have employed only hydrochloric acid in the hydrolysis and have found the concentration recommended by Good satisfactory as well as 2 to 2½ hours heating on a water bath.

The determination of the glucose resulting from the hydrolysis of glycogen may be carried out by most any of the well-known blood-sugar methods. When very large quantities of glycogen are present the authors have resorted to the Munson-Walker[21] gravimetric method in which the precipitated cuprous oxide is weighed. However, it is just as advantageous and possibly more expeditious to dilute the hydrolyzate until it falls within the range of the volumetric copper reagent available. We have found suitable for this purpose the Shaffer-Hartmann[22] blood-sugar method. Instead of using the values for blood sugar in the original work of Shaffer and Hartmann the authors have found it advantageous to compile data on standard glucose solutions and from these compose a graph from which the amount of glucose may be read in terms of cubic centimeters of standard sodium thiosulfate solution consumed. We consider the relation of glycogen to glucose to be one to one.

Total Carbohydrate Utilization. Many experiments have been conducted to determine the nutritional value of various carbohydrate-like substances in the rat. Pursuant to this end Cori and Cori[2] have employed a so-called carbohydrate balance sheet. The authors have repeatedly applied this experiment to the study of the nutritional value of many of the sugar alcohols in the white rat. The method as employed in this laboratory used fasted rats (48 hours) to which cacao butter was then given as food, this having been previously admixed with 33% of its weight of the carbohydrate-like substance in question. The feeding period was continued for 72 hours after which the animals were killed. During the feeding period the animals were kept in individual metabolism cages and the feces and urine carefully collected. The liver is rapidly extirpated under barbiturate anesthesia for glycogen determination in the usual manner. The alimentary tract is removed from the cardia to the anus for the determination of the unabsorbed carbohydrate. The carcass of the rat is cut into six parts with a cleaver, permitting admission to the tared vessel containing the 60% potassium hydroxide solution (about 100 cc.). The carcass is then weighed to the first decimal place. Cori used solid carbon dioxide to freeze the tissues of his animals after killing for the purpose of glycogen determinations. We have found this unnecessary as rapid manipulation with the cleaver permits the entire body to be placed in the alkali 60 seconds after death. The potassium hydroxide solution is heated on a water bath until solution is complete. This generally requires about one hour. The bones are removed by straining through cheese cloth and the solution is made up to a definite volume while hot. An aliquot portion is transferred to a centrifuge tube and the glycogen determined by the foregoing liver-glycogen procedure.

Having ascertained these data it is possible to calculate the percentage of carbohydrate utilized and absorbed. An example of a protocol taken from the authors' paper[9] on the utilization of inulin will illustrate the method of presentation of data. See Table XV.

TABLE XV

Utilization of Carbohydrates
Control Rats

Group No	No in Group	Wt of Rats, Gm	Food Ingested Type	Food Ingested Amount	Reducing Sugar after Hydrolysis, Tract, Gm	Reducing Sugar after Hydrolysis, Feces, Gm.	Wt. of Livers, Gm.	Wt of Tissues, Gm.	% Liver Glycogen	% Tissue Glycogen
1	2	138–73	Cacao Butter	12.5	.00	.00	6.90	161	.09	.20
2	2	150–147		14.1	.00	.02	8.90	234	.36	.11
3	2	102–101		12.4	.04	.05	8.20	165	.03	.09
4	2	107–97		10.5	.01	.01	6.80	171	.11	.10
5	1	108		8.2	.00	.03	3.50	78	.24	.04
6	2	115–148		17.5	.08	lost	8.50	192	.12	.15
								Mean	.16	.12

Experimental Animals

Group No	No of Rats	Wt of Rats, Gm	Food Ingested Type	Food Ingested Amount	Inulin in Feces, Gm	Inulin in Tract, Gm.	Inulin Equivalent of Liver Glycogen	% Liver Glycogen	% Tissue Glycogen	Inulin Utilized by Difference	% Inulin Utilized	% Inulin Absorbed	
1	2	120–122	Inulin from Arctium lappa and Cacao Butter	6.6	.43	.40	.29	3.11	.12	5.50	83.2	87.3	
2	2	125–125		6.9	.68	.46	.34	3.86	.16	5.45	78.6	83.3	
3	2	128–126		6.0	.24	.17	.24	2.79	.14	5.35	89.2	93.2	
4	2	122–124		5.5	.24	.23	.29	2.42	.08	4.73	86.0	91.4	
5	2	120–122		4.3	.11	.09	.20	2.20	.13	3.93	90.8	95.4	
								2.87	0.13		85.7	90.1	mean

FAT METABOLISM

Absorption of Fat. The percentage of fat absorbed from the alimentary tract 2, 4, 6, 8, and 12 hours after feeding was studied by Steenbock et al.[23] It was observed that partially hydrogenated vegetable oils were absorbed as readily as corn oil or lard. Furthermore, butter oil, halibut liver oil and cod liver oil were absorbed uniformly and at a more rapid rate than lard, corn oil or the partially hydrogenated fats. The method used for the determination of fat was that used by Cori[24] which consisted of killing the animals at the prescribed time interval and analyzing the alimentary tract for unabsorbed fat.

Fat Distribution. Reed et al.[25] made extensive studies of the distribution of fat in the various depots of the rat. More fats were stored by rats ingesting rations rich in fat than those on a diet rich in carbohydrate. The proportionate distribution of the stored fat was similar for rats of the same sex and weight regardless of the type of fat fed. Male rats of 250 grams weight stored more fat in the perirenal fat depot than female rats; the latter tended to store more fat in the genital fat depot.

The distribution of reserve fat in the female rat of 180 grams weight is proportioned as follows:

	Proportion of Total Fat
Subcutaneous	50
Genital	20
Perirenal	12
Mesenteric	10
Intramuscular	5
Omental	3

The fat distribution observed in normal rats is essentially the same in ovariectomized animals.[26]

The distribution of fat in the rat is independent of the type of diet fed; however, the amount of fat stored by the animal is greatest when a ration rich in fat is fed.

The method of fat estimation in the various tissues of the rats employed by these and other authors is set forth essentially as follows: the fat from each depot is preserved in 95% alcohol prior to saponification with alcoholic potassium hydroxide solution. From the soap formed by the hydrolysis the fatty acids are liberated by the addition of hydrochloric acid and extracted with ether. The ethereal solution of fatty acids is washed free of acid, dried with anhydrous sodium sulfate and filtered. Finally the ether is removed by distillation and the fatty acids dried to a constant weight and weighed.

A discussion, details and a summary of specific methods for the determination of fats in tissues and blood are given by Bloor.[27]

Fat Deficiency. McAnis et al.[28] have extensively studied the effect of fat-deficient diets on the rat. Animals were fed diets devoid of fat but containing adequate amounts of carbohydrate, protein, and vitamins. Comparatively good but not optimal growth rates were obtained for these animals. Animals receiving a diet containing a small portion of fat showed a much better growth. Burr and Burr[29] observed kidney lesions in rats fed a diet devoid of fat, which lesions are aggravated by a concomitant high-protein diet. Rats suffering from low-fat disease were not cured by feeding the saturated fatty acids, stearic, palmitric, myristic, lauric, or other lower acids. The same rats were cured by unsaturated fatty acids. They postulated that the body cannot synthesize appreciable amounts of linoleic acid which appears to be an essential fatty acid.

Burr and Belber[30] have shown that fat-deficient diets produced rats with a respiratory quotient greater than unity and exceptionally high metabolic rates after the feeding of carbohydrate. The animals apparently synthesize daily large quantities of fat but this autogenous fat does not prevent fat-deficiency symptoms.

Gregg[31] observed that rats fed a liberal fat diet showed only 22% more liver glycogen than completely fasted rats. No evidence to show that glycogen can be formed from fatty acids was given.

Ketosis. Deuel[6] and his co-workers fed rats the ethyl esters of many of the fatty acids. Those of the C_{2n-1} series, such as propionic, valeric, heptoic, pelargonic, and undecylic acids produced no ketonaemia. The esters of aceto-acetic, butyric, and caproic acid did produce blood ketone, more so than did the higher molecular-weight acids. They postulated that oleic, palmitic, and stearic acids break down into three fragments per molecule, each of which is capable of forming acetone.

NITROGEN METABOLISM

The fundamental studies on the nitrogen metabolism of the rat made early in the present century have withstood the test of time. Nitrogen distribution tables established many years ago for the rat remain for the most part unchallenged. The work of Cramer and Pringle[32] is given here as a reference for nitrogen exchange.

The distribution of the urinary nitrogen in young rats was investigated by Ordway and Morris[33] and a protocol from their work is given.

TABLE XVI

Nitrogen exchange in normal albino rats, diet—bread and milk

Period Days	Weight Gm	Weight of Nitrogen in Gm			
		Urine	Feces	Retention	In food
3	187	0.728	0.113	0 239	1 08
3	192	0.766	0.102	0.212	1 08
3	217	0 881	0 077	0 122	1.08

The extensive work of the Coris[2] on carbohydrate metabolism showed that in the fasting rat 90% of the total metabolism was due to fat oxidation. The remaining 10% was protein in origin. These data were based upon urinary nitrogen analyses.

In 1915 McCollum and Davis[34] showed that the lowest plane of protein intake derived from milk which can maintain young rats without loss of body weight is 3% of the food mixture.

Shortly thereafter Osborne and Ferry[35] demonstrated that economy in nutrition during the growth of the rat depends upon a correct adjustment between the proportion of protein and the total energy supplied. Furthermore, the optimum of protein is determined not only by the absolute amount of protein supplied the animal but also by its quality.

TABLE XVII

Protein metabolism in normal young rats

	Male Initial Wt 42 7 Gm. Final Wt. 41.0 Gm. Duration 9 Days		Urine Analysis, Male Initial Wt. 46.5 Gm. Final Wt. 51.5 Gm. Duration 15 Days		Urine Analysis, Female Initial Wt. 42.5 Gm. Final Wt. 39.0 Gm. Duration 10 days	
	Average for 24 hrs.	Average %	Average for 24 hrs.	Average %	Average for 24 hrs.	Average %
Total N	45 6	100.0	29 1	100 0	21.0	100.00
Urea N	32.5	71.3	22.2	76.3	16.5	78.6
Ammonia N	4.1	9 0	2.02	7.0	1 89	9.0
Uric Acid N	0.16	0.35	0.14	0.48	0 05	0.24
Creatinine N (C_1)	0.49	1.08	0.40	1 37+	0.34	1.42
Creatine N (C_2)						
$C_1 + C_2$	0.53	1.16	0.42	1.44	0.44	2 1

There are many papers extant in the literature on the determination of the adequacy of various proteins and the value of the individual amino acids in the rat's diet. The work by Rose and Cox[36] is typical of the procedures employed in the determination of the essential and non-essential amino acids. Rats were fed casein and their growth rates recorded. With completely hydrolyzed casein in the diet they grew to maturity but at a slower rate than those fed the unhydrolyzed protein. Rats fed upon the arginine-histidine-free amino acid mixture were neither able to grow nor maintain body weight, but promptly and continuously lost weight. The addition of histidine (1 Gm. per 100 Gm. of food) re-established growth among the rats fed this arginine-histidine-free amino acid mixture. This condition did not obtain with arginine.

In addition to the method of study of the essential nature of amino acids through the feeding of the hydrolytic products of various proteins, investigations using chemically-pure amino acid mixtures have been used extensively

TABLE XVIII

Final classification of amino acids with respect to their growth effects

Indispensable	Dispensable
Lysine	Glycine
Tryptophane	Alanine
Histidine	Serine
Phenylalanine	Norleucine
Leucine	Aspartic Acid
Isoleucine	Glutamic Acid
Threonine	Hydroxyglutamic Acid
Methionine	Proline
Valine	Hydroxyproline
*Arginine	Citrulline
	Tyrosine
	Cystine

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* Arginine can be synthesized by the animal organism, but not at a sufficiently rapid rate to meet the demands of normal growth

since 1930. By use of diets of this character the final classification of the amino acids with respect to growth in the rat has been established. (See Table XVIII taken from Rose.[37])

The relation of protein feeding to hemoglobin formation has been studied extensively by Pearson, Elvehjem, and Hart.[38] The animals in which a nutritional anemia had been induced were fed nine different types of proteins and hemoglobin regeneration was observed. The proteins of liver, casein, egg albumin, and soy bean oil meal were very effective in hemoglobin formation. In the rat there is no evidence that liver proteins are superior to other proteins of high quality in hemoglobin regeneration. Corn gluten meal and wheat gluten are poorly utilized by the rat both for growth and hemoglobin formation. Animals which were fed gliadin or gelatin which are inadequate for growth, rebuild their hemoglobin less rapidly than when fed a protein which permits growth.

RESPIRATORY METABOLISM

Determination of the Respiratory Quotient. On account of the ease ot standardization the rat is well suited for metabolism experiments and many laboratories have chosen the rat for this measurement and have contributed to the refined methods in use at the present time. Haldane's[39] open-circuit method continues to be the favored method of measurement of the R.Q. in most laboratories. Others have employed with success the closed-circuit or spirometer type of apparatus adapted to the rat.

The method employed in the authors' laboratory for many years has been the Haldane open-circuit method with certain modifications. The animals used in these determinations are young male rats weighing between 100 and 150 grams, fed a balanced laboratory ration, and kept in a dark room at a temperature of 26°C. ± 2°. Both 24- and 48-hour fasted animals have been employed; the latter giving more consistent results. The fasted animal is placed in the metabolism chamber and air is blown at the rate of 2 liters a minute through 2 scrubbing bottles of moist soda lime, No. 4 mesh, and one calcium-chloride absorber. The air is then passed through 2 concentrated sulfuric-acid water absorbers containing glass beads or marbles. In early experiments pumice which had been thrust into concentrated sulfuric acid after heating to a bright red according to the Haldane technic was used in place of the glass beads. The carbon-dioxide and water-free air is passed into the animal jar for 20 to 30 minutes, the exit tube being open to the outside air. After this period the metabolism chamber is closed, the air within having been adjusted to atmospheric pressure and a temperature of 28°C. The chamber and animal is weighed on a balance with a load capacity of 2 Kg. and a sensitivity of 1 mg. Weighings are to the third decimal place. After weighing, the metabolism chamber is inserted in the air line. The exit tube is connected with a series of absorption bottles containing sulfuric acid and glass beads for water absorption and moist soda lime for absorption of carbon dioxide. Check absorption bottles are kept in the chain and weighed with each determination. In this apparatus the efficiency of the water absorption apparatus is excellent. The soda lime requires moistening or replacing after every ten or twelve experiments. Five hundred cc. Woulff bottles were employed as absorbers and later 500 cc. Pyrex flasks with standard taper ground glass joints were used. All permanent connections were sealed with sealing wax.

No correction was made for the nitrogen content of the urine of the animals. This was deemed unnecessary as Cori and Cori have shown that the metabolism of the fasting rat is 90% fat oxidation.

Accuracy of the Determination. A series of determinations of oxygen consumption, heat production, and respiratory quotients of 92 fasting rats has been reported by Krantz and Carr.[40] An examination of the frequency distribution of the respiratory quotients of the rats of this series shows the median line practically identical with the mean of 0.725. The modal series falls within 0.720 and 0.729. Oxygen consumption fluctuated within very wide limits. Twenty-two % of the determination fell outside of the range, 150 to 250 mg. of oxygen per 100 Gm. of animal weight per hour with a mean of 185 mg. In later experiments the extreme variations in oxygen consumption have been eliminated by a more careful control of temperature and body weight of the animals. The results of recent experiments are shown in Table XIX.

Factors Influencing Gaseous Metabolism. Horst et al.[41, 42] have shown the metabolism of the rat to be subject to diurnal variation. The oxygen uptake is high in the morning and high after 4:00 P.M., the values sometimes ranging from 13 to 50% greater than during the middle of the day. They therefore suggest that metabolism experiments be conducted between 10:00 A.M. and 4:00 P.M. Davis and Hastings'[43] experiments indicate that the oxygen consumption of rats decreases with age up to 4 months. No difference was found between the oxygen consumption of males and females in this series of 136 animals. Benedict and MacLeod[44, 45] list a complete bibliography of all of the methods employed in the metabolism of the rat. These workers showed that on the average the heat production of 4-½ month old rats decreases during the first 24 hours of fasting approximately 10%. With rats younger than 4 months, the metabolism decreases an average of 28% during 24 hours of fasting. The same investigators[44, 45] studied the influence of temperature, season, and sex on metabolism. They observed that the critical temperature for basal metabolism is 28°C., the heat production is 10% lower in the summer than in the winter and male rats have a definitely higher metabolism than female rats up to 14 months of age. Sherwood[46] made the observations from studies on 2000 determinations on normal rats that animals weighing approximately 100 grams exhibited a 26% greater diminution in metabolism during the summer months. Larger animals likewise experienced a decrease in metabolism in the summer but of a lesser degree than that exhibited by the smaller animals. It is of interest to note that this 26% diminution in metabolism is magnified when expressed as calories per Kg., the value becoming 42%. Sherwood is of the opinion that the weight technic is slightly

TABLE XIX

Metabolism of the fasting albino rat measured by the Haldane method

Series		Body Wt Gm	Body Temp °F	Gm O^2 per 100 Gm Body Wt per hr	Calories per Sq M per 24 hr	R Q		No. of Rats	No. of Determinations
						48 hr Fasting	Non-Fasting		
	Mean	170	. .	0 183	1040	0 725		92	92
A	Range	120–185	. . .	0 129–0 266	772–1505	0 682–0 767			
	Sigma			0 032	170	0 021			
	Mean	143	. . .	0 189	1119	0 715		29	29
B	Range	91–177	. . .	0 143–0 268		0.620–0.760			
	Sigma			0 028		0 023			
	Mean	161	99 3	0 181	1169		0.894	11	68*
C	Range	120–201	97.2–101 8	0 150–0 211	993–1450		0.754–1.072		
	Sigma			0.016	104				
	Mean	131	. . .	0 191	1096	0 714		45	45
D	Range	108–163	. . .	0 155–0 233	892–1341	0.688–0 735			
	Sigma			0 019	118	0 012			
	Mean	129	. . .	0 169	970	0 725		32	32
E	Range	112–154	. . .	0 129–0 206		0 683–0.748			
	Sigma			0 017		0.013			
	Mean	125	. . .	0.177	1002	0.733		49	49
F	Range	93–164	. . .	0 120–0.221		0 695–0.767			
	Sigma			0 022		0 018			

* 22 of these determinations were made on 4 rats by the closed chamber method

more reliable than the surface area technic when measured by the Diack formula.

Horst et al.[41, 42] studied the influence of the character of the diet prior to metabolism determinations. They observed that many types of balanced rations gave essentially uniform results. A diminished protein intake prior to the experiment generally showed a reduced metabolism in the animal.

In a study of 282 determinations made on 9 female rats during the stages of estrus Lee[47] found that there was a statistically significant increase in the heat production of animals during the last 10 hours of diestrus and the first 6 hours of proestrus. No change in the metabolic level was found in any other phase of the cycle.

EFFECT OF OPERATIVE PROCEDURES UPON METABOLISM

Smith et al.[48] observed that the administration of thyroid to thyroidectomized and hypophysectomized animals increased the metabolism to a greater degree than when administered to normal animals. Each operative procedure was found to lower the metabolism of the rat. Russell[49] showed that in the hypophysectomized rat glucose oxidation and heat production are much higher than in unoperated animals. Hypophysectomized rats, having access to ample carbohydrate supplies showed a higher respiratory quotient than did normal animals under the same conditions. The experiments of Carr and Beck[50] showed that after bilateral adrenalectomy in the rat there is a reduction of approximately 25% in the total metabolism. Following the consumption of carbohydrate the respiratory quotient of the adrenalectomized rat is similar to that of the normal animal. Ashworth and Cowgill[51] in the study of the adrenalectomized rat weighing 100 Gm. found a diminution of 40% in metabolism without any appreciable effect on the endogenous nitrogen excretion.

Hemmingsen[52] studied extensively the effect of spaying on the metabolism of female rats as compared with the metabolism of their unoperated sisters. He concluded that if an actual difference in gaseous exchange does occur, it is lower in the operated animals and here extraordinarily small, i.e., approximately one per cent.

EFFECT OF THE ADMINISTRATION OF DRUGS UPON METABOLISM

The new alkaloid from ergot, i.e., ergonovine was shown by Davis et al.[53] to increase the metabolic rate of rats as much as 80% when administered intravenously in doses of 10 mg. per Kg. Terada and Tainter[54] administered alpha dinitrophenol to normal rats and observed a marked increase in metabolism which was a function of the dose administered. Carr et al.[10] measured the oxygen consumption of the fasting rat and found that it was increased approximately 32% by the subcutaneous injection of epinephrine. The respiratory quotient was unaltered. This increase in metabolism was not antagonized by injections of insulin-free pancreatic extracts which counteract the pressor action of epinephrine.

Chevalier[55] administered vitamin A in oil solution to rats and found that the gaseous metabolism diminished with the increase of the vitamin ingested. With 2000 units daily a reduction of approximately 16% was observed. Injections of the chlorophyllate of sodium were shown by Rydin[56] to have a thyroid-like action on the rat augmenting its metabolism. The injection of Nembutal was found by Krantz et al.[57] to diminish the oxygen uptake of the rat. Picrotoxin significantly antagonized the diminished oxygen-consumption of the animals in which Nembutal had been injected. Picrotoxin, however, does not augment the oxygen uptake of normal rats.

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9

The Central Nervous System

By

W. A. JEFFERS and J. Q. GRIFFITH, Jr.

Assisted by E. Roberts

In this chapter are outlined those procedures found valuable in the experimental study of the rat's central nervous system, either in regard to its normal anatomy and function, or in relation to various types of brain lesions. It will be apparent that most of them are only a part of more extensive experiments designed to test the function of the central nervous system as it affects other organs. Where useful alternative methods have been tried, they are indicated by reference only.

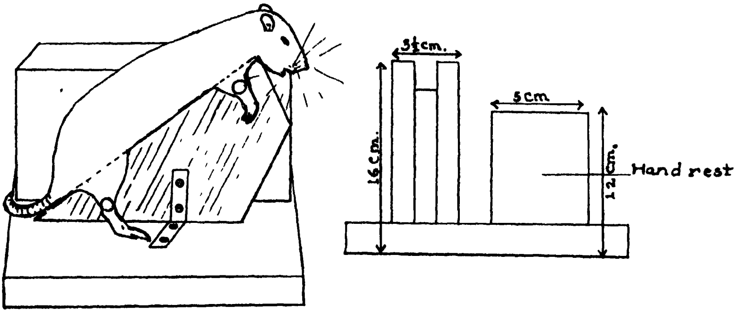

Fig. 110. Stand for cisternal puncture.

CISTERNAL PUNCTURE

The rat, anesthetized, is placed prone over a stand (Fig. 110) with head elevated so that the long axis of the body lies at a 50° angle with the horizontal, and the four paws are secured with thumb tacks to maintain this position. The head is allowed to hang over the upper part of the stand, and is freely movable. The hollow center of the stand is designed to prevent compression of the trachea.

Upon flexing the head acutely, the external occipital protuberance is thrown into prominence. Directly caudal to this a depression is felt between the protuberance and the spine of the atlas. The needle is thrust into the center of this depression. As it enters the cisterna magna there is felt a sudden decrease in resistance, and the cerebrospinal fluid will rise in the glass tubing, which has been partially filled with physiologic saline solution prior to the puncture. Rubber tubing may be connected with the glass to facilitate aspiration or injection.

Character of the Cerebrospinal Fluid of the Rat. Fluid withdrawn by cisternal puncture is clear and colorless. A smear stained with Wright's stain will show an occasional mononuclear cell. Signs of meningeal irritation to be observed after the intracisternal injection of material such as kaolin include leucocytosis, with polymorphonuclear forms predominating, and an increased protein content.

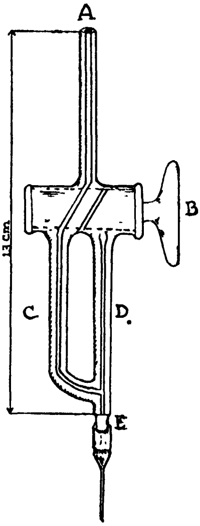

Fig. 111. Pipette for cisternal injection.

A roughly quantitative estimation of the degree of increase in protein can be made by the simple method of Mestrezat and Magitot.[1] Known dilutions of the protein of egg albumin or blood serum are placed in a hanging drop slide over a dark background. To each is added a drop of 10% trichloracetic acid. A drop of cerebrospinal fluid is treated likewise, and the flocculation so produced is compared with that of the known protein standards.

Method for Intracisternal Injection. The apparatus is shown in Figure 111. A rubber mouth-piece fits over end A for suction or expulsion of fluid. Through stopcock B either capillary tube C or D may be connected. E fits snugly into a hypodermic needle ⅝″ long. The fluid to be injected is first drawn up through E into the very lower part of tube C. Stopcock B is then turned and tube D filled with fluid nearly to the top. Stopcock B is then turned to a neutral position and cisternal puncture performed as just described. When it is thought that the cistern has been entered stopcock B is turned so as to make connection with C; gentle suction is employed, and the cerebrospinal fluid, mixed with a little of the injection fluid, rises in C. The stopcock is then turned to connect with D, and the column is then expelled with gentle force until approximately the same amount originally withdrawn is replaced. The needle is then withdrawn.

Cerebrospinal Fluid Pressure. Ether or Nembutal anesthesia may be used. The method consists of balancing the pressure of the cerebrospinal fluid against a known pressure by means of a bubble manometer. This device is illustrated in Figure 112. A-B is a thick-walled glass capillary tube, which contains the bubble. Below it rests a scale for locating the bubble. At one end the capillary tube is connected by a length of heavy rubber tubing with a globular glass container H. This container may be raised or lowered manually by a pulley system and the height of its fluid contents read against an opposed centimeter scale I. At the other end the capillary tube is connected through a stopcock C with either a tuberculin syringe D or a needle used for cisternal puncture (see technic for cisternal puncture). E and F are heavy-pressure rubber tubing, while G is a flexible brass tube with an adapter to fit the needle (heavy rubber tubing could be used but is less convenient).

In making the measurement, distilled water tinted with methylene blue is used. Container H is filled and refilled as needed without permitting the fluid level to fall at any time below the bottom of the container. The stopcock is turned to connect with tube F, the syringe D not yet being attached. Syringe D is filled separately one-quarter full with the tinted distilled water. Then, when flow through F is free and without bubbles, the tube F is fastened to syringe D and the syringe permitted to fill to the three-quarters mark with fluid from reservoir H. There must be no air bubbles in the system. The stopcock is turned to connect with G and free flow permitted through the needle for several minutes, until it is certain that there are no bubbles remaining. Then the stopcock is turned to a neutral position, so that the capillary tube A-B is connected with neither F nor G. Tube J is pinched shut and removed from the end of the capillary tube, the stopcock C turned to connect with syringe D and the piston drawn out so that an air space, about 3 to 4 mm. long, appears in the B-end of the tube. Tube J is then released and while fluid is flowing from its end it is again inserted over B. The piston of the syringe is now drawn out further until the bubble is approximately at the center of the Tube A-B. Stopcock C is again turned to a neutral position, reservoir H lowered, and cisternal puncture attempted. The animal is pinned on a board lying on one side with its sagittal plane parallel

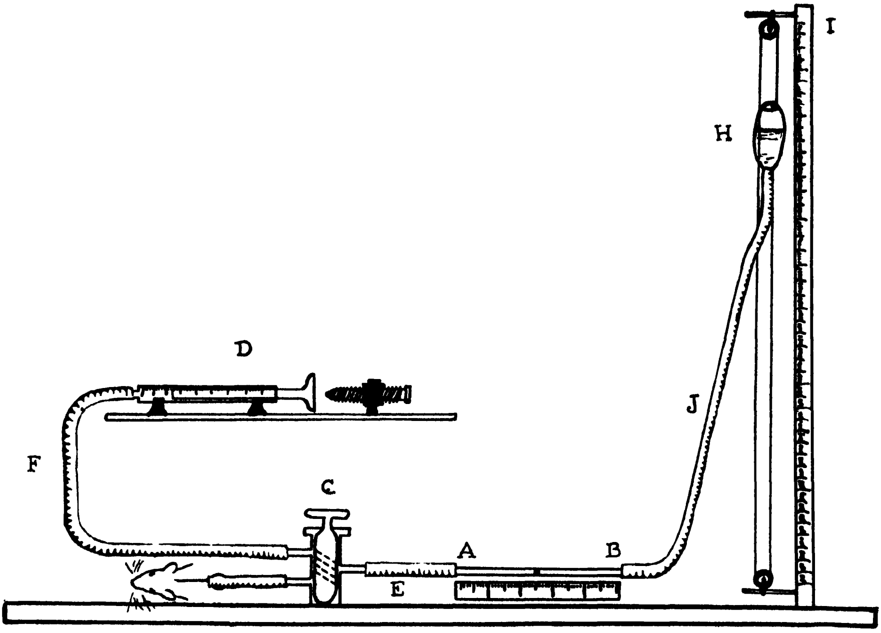

Fig. 112. Diagram of set-up for measuring cerebrospinal fluid pressure.

with the table. When the point of the needle approaches the cistern, stopcock C is turned to connect the needle with the manometer, and an assistant watches the bubble. As soon as the cistern is entered the bubble moves toward B (i.e. away from the needle), and it is necessary to raise and adjust reservoir H quickly to such a point that the bubble is motionless, except for slight respiratory excursions, at its original position. The reading on scale I, less the height of the needle above the base of scale I, is the cerebrospinal fluid pressure. At the end of the determination, stopcock C is again turned to a neutral position, and the needle withdrawn. If a series of determinations on different animals are being done, the needle should be cleaned as follows: Turn stopcock C so the syringe D is connected with the capillary tube. Using the screw mounting on the piston, force the bubble from its mid-position over toward B. Turn stopcock C so as to connect with G and the needle and let the bubble go nearly to A before shutting off the stopcock. This expels a drop of fluid from the needle. This maneuver may be repeated several times.

This procedure requires good team work and a certain amount of speed. Once the setup is ready and the animals anesthetized, it is quite possible to make the measurement on 6 animals in a half hour. Results are quite consistent. Most animals have pressures ranging from 60 to 80 mm. of water. We have never seen a normal animal with a pressure over 100. In pathologic states, as after intracisternal kaolin injection, the pressure may reach 250 to 300 mm.

METHODS FOR PRODUCING HYDROCEPHALUS

Intracisternal Injection of Kaolin. Rats which have been injected with kaolin intracisternally and subsequently have developed a hypertension will, if they survive 2 or 3 weeks, usually develop an internal hydrocephalus (see Fig. 113). This is a communicating hydrocephalus because: (1) cisternal puncture reaches cerebrospinal fluid which is under increased pressure; and (2) thorotrast injected into the cistern and followed by roentgenogram, passes both into the subarachnoid space (encephalogram) and into the ventricle (ventriculogram).

Intraventricular Injection of Mineral Oil. This has been described by Lashley and others.[2] A trephine hole is made in the middle of the left frontal bone, and through it a hypodermic needle is passed into the lateral ventricle. Sterile paraffin oil, at body temperature, is then injected through a hand syringe until respirations cease (usually this requires 200 to 400 cu. mm.). The injection is then stopped and the animal recovers. Hydrocephalus develops involving the entire ventricular system. We have employed this technic in six animals with identical results. We have found it more convenient to dispense with the trephine opening and simply thrust the needle through the skull cap. We insert it somewhat to the side and pass it along parallel with and about 4 mm. below the skull cap to a point midway between the side of the head and the superior longitudinal sinus. For orientation a cross section should be cut through the skull and brain, after formalin fixation, to show the location of the lateral ventricle.

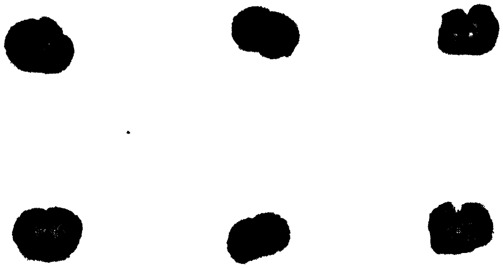

Fig. 113. Brains of rats made hydrocephalic by the intracisternal injection of kaolin or by growing occipital tumor. The normal brain is in the middle. The upper and lower sections in each column are from the same brain, the upper being the more anterior.

METHOD FOR PRODUCING SUBARACHNOID HEMORRHAGE

Under any anesthetic an incision is made through the skin overlying the skull to expose the longitudinal or lateral venous sinuses (see Chapter 3). The lateral sinuses lie, on each side, just anterior to the transverse ridge at the junction of the parietal and interparietal bones. This ridge is immediately anterior to the posterior fourth of the cranium. The sinuses can be seen faintly through the thin skull cap. The longitudinal or sagittal sinus lies directly beneath the sagittal suture. A cutting-edge needle is thrust through the skull directly over one of the sinuses and is made to pass onward for a distance of approximately 3 mm. This pierces the sinus. Free bleeding occurs externally and also into the cerebrospinal fluid, as may be confirmed by cisternal puncture.

METHOD FOR DIFFERENTIATING OLD FROM RECENT HEMORRHAGE IN THE CEREBROSPINAL FLUID OF RATS

By E. Roberts

This method was originally reported by Griffith, Roberts and Jeffers.[3] A thin film of spinal fluid containing red cells is prepared upon a cover slip as if for a differential blood count and allowed to dry in air. This requires 15 to 30 minutes. It is then immersed for 20 minutes in 50% alcohol saturated with Sudan III. It is thoroughly rinsed in several changes of distilled water, requiring 3 to 4 minutes, and is blotted on filter paper (it is never permitted to dry in air), and is mounted in glycerin and examined immediately. If the red cells are present as the result of fresh hemorrhage they will appear as homogeneously staining, yellow disks. If the hemorrhage is 24 hours or more old practically all the cells will show as faint rings with clear unstained centers. If the hemorrhage is 4 to 6 hours old most of the cells will be of the fresh type, but a few of the older forms with unstained centers will be present.

BRAIN TUMORS

Implantation. Rats* bearing a sarcoma were anesthetized with ether and the growing tumor exposed by operation.

The skull of the recipient rat, likewise anesthetized, is trephined over the region into which the tumor is to be implanted, the hole being made only large enough to admit a glass capillary pipette having a bore of about 1 mm.

A piece of fresh tumor tissue about 1 mm. square is held against the tip of the pipette by gentle suction. This is thrust deeply into the brain tissue and expelled by positive pressure. A plug of blood clot will seal the trephine opening, preventing the tumor tissue from being discharged. The skin wound is then closed with a silk suture.

The donor rat's tumor can be used for about a dozen brain implantations. The tumor can be kept indefinitely by transferring it to tumor susceptible rats.* With the same susceptible rats, brain tumors of obstructive size will appear in 2-4 weeks in 60% of the rats so treated.

Irritation with Carcinogenetic Substances. This method was reported by A. Weil.[4] A compound known as "Styrly 430" produced tumors in 3 out of 5 rats in 4 months. Other preparations were less successful.

TECHNIC FOR LABYRINTHECTOMY

This was described by Yueh T'ang and Chen-Fu Wu.[5] Under ether anesthesia the rat is fixed in the supine position. The common carotid on the side to be operated is ligated, to prevent excessive bleeding. The hair of the neck is clipped short. An incision is made 1.5 cm. long a little to one side of the median line, beginning 8 mm. rostrad to the angle of the lower jaw and extending caudad. The tissues are divided by blunt dissection until the bulla ossea is seen; this lies medial to the angle of the mandible. In the ventral wall of the bulla an opening is made. With a fine lamp and loop (lens) the promontory over the cochlea is located and this determines approximately where the non-auditory labyrinth lies. Then the labyrinth is destroyed with a small chisel, and the wound sutured.

METHOD FOR OBTAINING A DECEREBRATE PREPARATION

The method given is that described by Landis,[6] though a more detailed and complicated technic is given by Bell, Horne, and Magee.[7] Under ether anesthesia, the carotid arteries are ligated and the trachea cannulated. The top of the cranium is removed and the cerebral hemispheres carefully scooped out. Sometimes the blood in the superior longitudinal sinus must be coagulated by a cautery. The loss of blood is usually slight.

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* "Obtained from the Rockland Farms."

PRODUCING BRAIN LESIONS IN RATS WITHOUT OPENING THE SKULL

This method was described by Dennis, and Bolton.[8] Under ether anesthesia the skull is exposed and the cautery applied, for about 50 seconds. This coagulates the underlying brain tissue. The wound is sewed and covered with collodion.

METHOD FOR REMOVING THE FRONTAL LOBES OF THE BRAIN

This method was described by Richter and Hawkes.[9] A midline scalp incision is made 1 cm. long exposing the frontal and parietal sutures in the skull of an anesthetized rat. After scraping the fascia from the bone, a circular hole 2 mm. in diameter is made with a dental trephine just posterior to the frontal suture. Care must be taken not to tear the meninges. The meninges is next opened by piercing with a needle, and through this opening a glass cannula with bevelled edge is inserted. The cannula is connected with a faucet suction pump and the brain tissue sucked out by negative pressure. In order to avoid the bleeding which almost invariably results from tearing the meninges, the cannula is inserted several millimeters into the brain tissue before the suction is applied. By moving the cannula it was found possible to remove a fairly constant amount of brain tissue in every animal. The animals required considerable attention postoperatively.

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10

Technics for the Investigation of Behavioral Phenomena in the Rat

By

GEORGE L. KREEZER

INTRODUCTION

THE present chapter is intended to furnish an outline of technics available for investigation of behavioral phenomena in the rat. In the arrangement of topics, a handbook plan of organization has been adopted. Various phenomena of behavior prominently represented in the experimental literature have been used as main headings and presented in alphabetical order. When a given technic falls under more than one heading, cross references are used. To facilitate the location of special technics, an index to the section headings is given in the table of contents.

In the organization of the individual sections, the guiding schema has been the representation of a given phenomenon as a function of various factors. The phenomenon has the status of the dependent variable and the factors the status of independent variables. In terms of this schema, a suitable outline of technics may be expected to furnish a guide to procedures available for description and control of both the dependent and independent variables. The technics available for measurement of the dependent variable, the behavioral phenomenon under consideration, have been described with sufficient fullness to indicate the essential features. A guide to the technics dealing with the independent variables has been provided by way of a classification of the literature concerned with special problems. These problems are formulated, in the main, in terms of the formula representing the behavioral phenomenon as a function of various factors, with the factors, and their associated references, listed under a given rubric in alphabetical order. The classifications of the literature are given at the end of the special sections.

With respect to the temporal scope of the bibliography, advantage has been taken of the fact that Munn[433] has published an excellent survey of the literature up to August, 1932. In the present survey, references to papers already cited and summarized in Munn are not, in general, included. Munn's monograph should therefore be consulted for the earlier literature. In the description of the basic technics, however, this limitation has not been observed since it was considered desirable that the survey of such technics be reasonably complete. The chief source of the titles in the bibliography has been those indexed for the rat in Psychological Abstracts from 1932 through December, 1939. A number of additional references which have come to the writer's attention have been included, but no thorough independent search for additional titles has been made. In addition to Munn's monograph, other general works on comparative psychology which should be of value to the reader, even though they are not limited to material on the rat, are those of Warden, Jenkins, and Warner,[628] Moss,[418] Washburn,[632] and Maier and Schneirla.[382a]

ACTIVITY LEVEL

A noteworthy characteristic of the rat's general behavior is the presence of a considerable degree of movement and activity which is not immediately explainable in terms of specific stimulus conditions or incentives provided by the experimenter. It has been natural therefore for inquiries to be made concerning the extent of this general activity, the conditions inside and outside of the animal on which it may depend, and its possible relation to the animal's specific needs and drives. In the present section are considered the methods available for measuring the general level of activity of the rat, and the means by which various conditions which may influence this activity may be investigated.

Apparatus for Measuring General Level of Activity. The three chief types of apparatus which may be used for measuring the general level of activity in the rat are (1) the vertically revolving drum; (2) the horizontally rotating turn-table; and (3) the tambour or spring-mounted activity cage. These types of apparatus have been used in a fairly wide variety of forms, and special forms have been devised for measuring the amount of specific kinds of activity, such as eating or drinking. Surveys of different types of apparatus available in this general field have been made by Syzmanski,[572] and by Richter.[475]

Revolving Drums.[200, 475, 494, 531] In apparatus employing a vertically revolving drum, it is customary to have beside the drum a small cage in which the animal may live when not running in the drum. In some forms of this apparatus a sliding door may be closed between the drum and the cage so that the rat may be forced to remain either in the drum or in the cage. The number of revolutions of the drum produced by the running of the animal may be totalled on an automatic counter or a graphic record made on some other form of recording apparatus. One example of this type of apparatus is illustrated in Figure 114.[*] A study of the reliability of the activity wheel technic has been made by Shirley[525] and a useful analysis of factors involved in its use by Skinner.[531]

[*] All figures except 114 and 120 have been redrawn on the basis of original figures in the references cited. When the figure is specified as redrawn, some slight modification has been made. Thanks are due Mr. Franklin W. Smith for the drawings.

Turn-table. A turn-table which rotates in an approximately horizontal plane has been used at the Wistar Institute in a manner similar to the revolving drum. This affords an inexpensive device on which rats may exercise. By the attachment of a counting or recording device, the turn-table has been adapted for measurements of level of general activity as described in Chapter 1. Descriptions of turn-tables have been given by Greenman and Dührung,[200] and by Farris and Engvall.[165]

Activity Cages.[572, 475, 474, 647, 277] In this type of apparatus, the cage in which the animal lives is suspended by means of rubber tambours or springs so as to be freely movable. Movements of the rat are transferred to the cage and then, by means of air pressure changes or other mechanical devices, to a writing point recording on a smoked drum or other type of recording surface. A diagrammatic illustration of a tambour-mounted activity cage described by Richter is shown in Figure 115.[475]

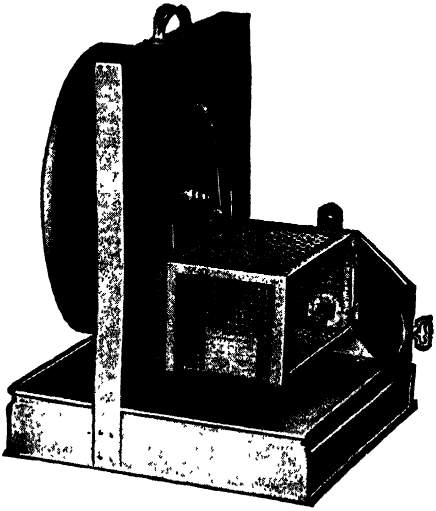

Fig. 114. A Vertically Revolving Activity Drum.

Living cage is rectangular enclosure at right side of apparatus Rat may pass from it to the vertical drum. The number of revolutions of the drum is indicated on a counter [Courtesy Geo H. Wahmann Mfg. Co , Baltimore]

Conditions Regulating Level of Activity. A considerable variety of investigations has been performed for determining the conditions on which the general level of activity in the rat may depend. For measuring the dependent variable, the level of activity, devices such as those just described have been used. For controlling the independent variable, some special technic appropriate to the particular condition must be employed. A list of the variety of conditions the effects of which on the rat's activity have been investigated are classified below together with references in which may be found descriptions of the technics used.

I. *Biological factors**
  A. *Bio-chemical factors:*
    1. Drugs[445, 519, 677]
    2. Endocrine factors:
      (a) Adrenal hormones[481]

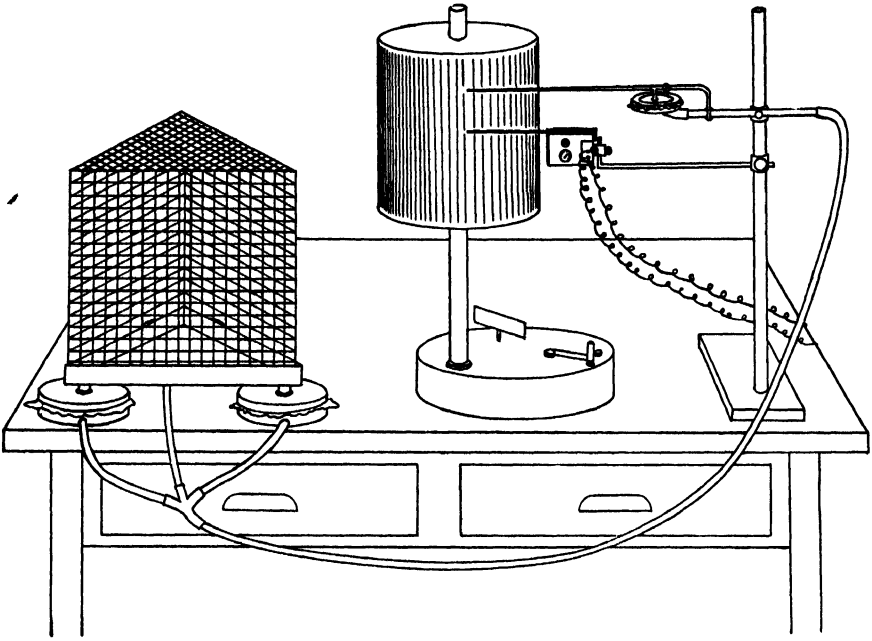

Fig. 115. A Tambour-Mounted Activity Cage.

The three corners of the cage are mounted on rubber tambours Movements of the rat in the cage are transmitted by air-pressure changes to the writing-point, which leaves a record on the smoked drum A second writing-point provides a time-record [Redrawn from Richter[475] Animal Behaviour and Internal Drives, Quart Rev Biol, **2**: 307–343, 1927]

      (b) Female sex hormones[488]
      (c) Pituitary hormones[486, 477]
      (d) Testicular hormones[236, 479, 480]
      (e) Thyroid hormones[478, 215]
    3. Nutritional and metabolic factors[509, 304]
  B. *Developmental factors*[508, 198]
  C. *Digestive functions*[493, 485]
  D. *Neural structures*[489]
  E. *Pathological states*[676]
II. *Psychological factors*[540, 152, 278]

DISCRIMINATIVE CAPACITIES

For the investigation of the discriminative capacities of the rat, three types of method are applicable: (1) the stimulus-test method; (2) the discrimination-response method; and (3) the conditioned-response method.

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* In the classification of factors into biological and psychological factors in this and later sections, the implication is not intended that psychological factors are not also biological. The heading "biological factors" may be regarded as equivalent to the caption "non-psychological biological factors."

The stimulus-test method has been used only with fetal and new-born rats. The method involves attempts to stimulate the animal in various ways, and the observation of whether or not responses occur. Thus the fetal rat may have pressure hairs applied at various points of the body surface, light flashed in the direction of the eyes, or a tone sounded in the ears and the presence or absence of a response taken as an index of whether or not the animal is sensitive to the stimulus in question. The experiments must, of course, be conducted with appropriate controls in order to justify the conclusion that the movements observed are functions of the stimulus and are not a matter of chance. Illustrations of the use of the technic with fetal animals may be found in papers of Angulo y Gonzales,[16, 17, 18] Raney and Carmichael,[466] and Windle and co-workers.[654] Its use with new-born animals is reported in a paper by Anderson and Patrick.[7]

The discrimination-response and conditioned-response methods depend on the animal being trained to move in a particular way in response to one of two or more stimuli, and not to make that response to the other stimuli. The consistent differentiation of the animal's response in relation to the different stimuli is taken as evidence of his capacity to discriminate them. Common to the two methods is the provision for some motivation or drive on the part of the animal which will make it possible for it to be trained to respond in a particular way to the stimulus chosen by the experimenter as positive.

The most obvious difference in the nature of the discrimination-response method and the conditioned-response methods lies in the temporal-spatial relations of the stimuli. In the discrimination-response method, two stimuli or stimulus-objects are presented simultaneously and in different spatial positions. In the conditioned-response method, the stimuli to be differentiated are presented in temporal sequence and, consequently, need not show spatial differentiation. In their early forms, the two methods tended also to differ in the nature of the animal's response. In the discrimination-response method, a correct response involves a locomotor movement of the entire animal. In the conditioned-response method, the differentiating response involves movement or activity of only a particular part of the animal. The animal remains in the same general locality throughout the series of stimulus trials. In recent forms of the two methods, as used with rats, the distinction in terms of the mode of response required has tended to become less marked. Both methods may be used in a variety of forms. Details concerning the two methods are given below.

(*a.*) *Discrimination-Response Method*

The nature of the discrimination-response method may be illustrated by reference to the Yerkes light-discrimination box shown in Figure 116.[333] The chief parts of this discrimination box are an entrance compartment S, the discrimination chamber D and food compartments F and F′. The discrimination chamber D is divided into two alley-ways. At the end of each of these is a window or frame by means of which a visual stimulus-object of any desired properties may be presented. Thus a bright area may be present at the end of one passageway and a dark area at the end of the other. A movable screen which may be shifted from side to side in the light box L, located behind the windows, provides for differences in illumination. Wire grills G and G′, on the floor of the alleys, make it possible to shock the animal when it runs down an alley arbitrarily selected by the experimenter

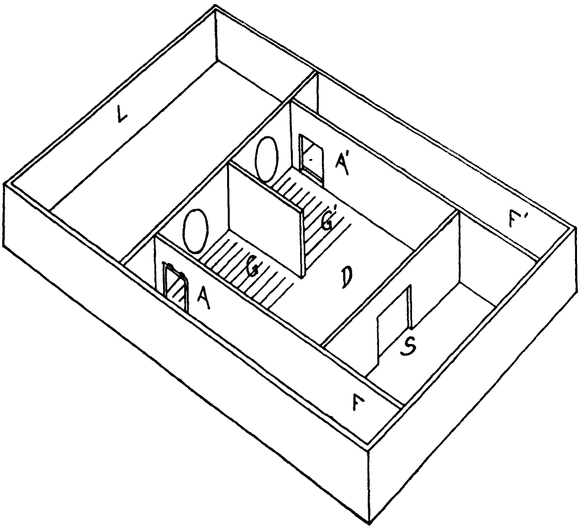

Fig. 116. Yerkes Brightness-Discrimination Box.

S, starting chamber, D, discrimination chamber, G, G′, electric grills for administering shock; A, A′, doors to food boxes F and F′, L, light box. [Redrawn from Lashley[338] Brain Mechanisms and Intelligence. By permission of the publishers, University of Chicago Press.]

as negative. Doors A and A′ near the ends of the alleys permit the animal to escape into the food compartments.

In a typical trial, a hungry rat is released into the discrimination chamber D from the entrance box. Assume that a bright area has been arbitrarily selected as the positive, or correct, stimulus-object. The grill of the other, negative, alley is therefore charged and the corresponding door kept locked. If the rat runs into the corresponding alley, it receives a shock as it crosses the grill, and is thus induced to retrace its path. If the rat runs down the other alley, toward the positive stimulus, no shock is given and it is permitted to find the door A into the food box F. After a large number of such trials, in which the rat is punished by shock upon entering the "negative alley" and rewarded by food upon entering the "positive alley," the rat may learn to run consistently toward the positive stimulus and through the corresponding door to the food box, even though all other factors, such as relative positions of the positive and negative alleys, cues from smell, etc. are systematically varied by the experimenter. An arbitrary criterion is used as an index of when the animal may be regarded as having learned to run directly to the food box—e.g., a succession of 30 to 50 correct trials.

The Yerkes light-discrimination box may be regarded as a basic type of apparatus from which most other forms of discrimination apparatus have been developed. These other forms of apparatus have been introduced to meet two types of need: either that of increasing the efficiency of the method, as through reduction of the number of trials required for the animal to learn to make a discrimination, or of making possible tests for discrimination of a variety of different properties of stimulus-objects. Thus in order to increase the efficiency of the discrimination-method as represented by Yerkes discrimination box, modifications have been introduced successively by Fields, by Lashley, by Munn, and, most recently, by Morgan. In order to permit tests of discrimination to be made for different types and properties of stimulus-object, a considerable variety of special modifications have been found necessary. A summary is given below of conventional practice with respect to various conditions involved in the use of different basic forms of the discrimination-apparatus.

Yerkes Discrimination Box

(1) **Apparatus.** The essential features are illustrated in the brightness discrimination box. The plan and description of the use of one form are given above. The method was originally described by Yerkes in 1907.[663] An improved form for investigation of visual sensitivity was described by Yerkes and Watson in 1911;[665] this is essentially the same form illustrated in Figure 114, which was taken from Lashley.[333] Lashley described a modified form in 1935;[337] the stimulus-cards were mounted on swinging doors, which the animal had to push aside to get to the food-box. This modified design permitted the initial training series to be reduced from an average length of 125 trials to 67 trials.

(2) **Motivating Factors.**

(a) *Drive:* Hunger

(b) *Reward (or incentive) for correct response:* Brief feeding period in food box.

(c) *Punishment for incorrect response:* Electric shock as soon as animal partly or wholly crosses the grill.

(3) **Training Procedure.**

(a) *Preliminary habituation:* Rat placed in box with all doors open and no special stimuli present and permitted to explore apparatus for period of half an hour on 5 days before regular training period begins.[337]

(b) *Criterion of mastery of discrimination task:* Ranges from 80 to 100% correct responses in a total of 20 to 50 trials.[198, 433]

(c) *Trials per day:* 10 trials per day is representative practice.[337]

(d) *Trials required for mastery:* Varies with nature of discrimination to be made. Indicative figures are: For discrimination between illuminated and dark areas, 125 in 1929 Lashley apparatus and 67 trials in 1935 Lashley apparatus.[337]

(4) **Criticism of method:** See Fields.[166]

(5) **Modifications:** Borovski[49] and Stone[553] have described multiple discrimination boxes, which may be regarded as equivalent to a number of simple Yerkes discrimination chambers arranged in series.[433]

Fields' Discrimination Box[166]

On the basis of his criticism of the Yerkes discrimination apparatus in tests of pattern-discrimination, Fields devised an apparatus which led to a reduction in the number of trials required to establish a given discrimination. The chief changes from the Yerkes box consisted in the elimination of the partition which separated the space in front of the two stimulus-objects, the elimination of the electric grills for shocking the rat and the bringing of the food-reward into more direct relation to the positive stimulus-object.

Since Fields' discrimination box has been supplanted by other still more efficient forms, further details need not be given here. His criticism played an important rôle in initiating the series of attempts made to evolve more efficient forms of discrimination-apparatus.

Lashley Jumping Technic[334]

(1) **Apparatus.** The rat is trained to jump against a card bearing a stimulus-pattern of some sort from a stand located a short distance in front of the card. Details of the apparatus are shown in Figure 117.

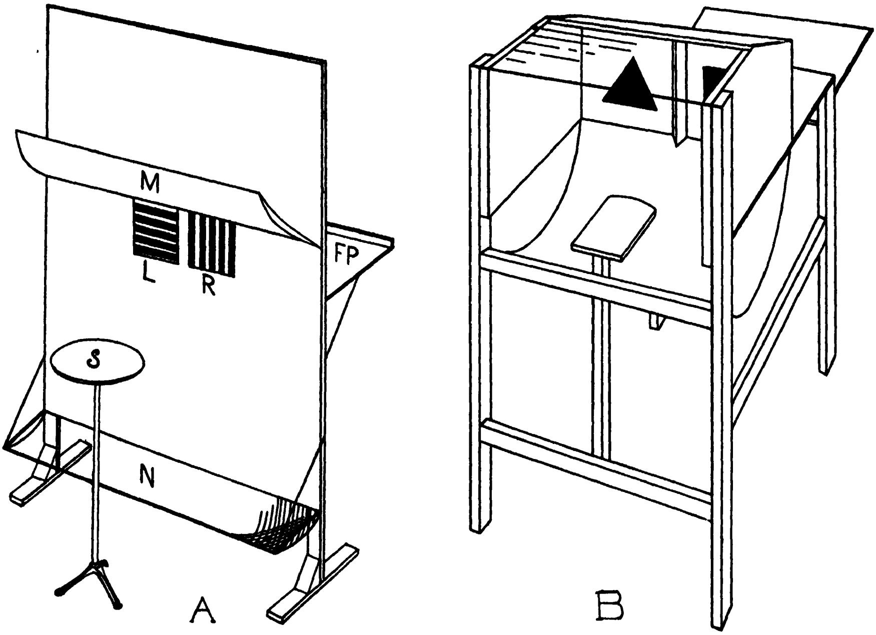

Fig. 117. Lashley Discrimination Apparatus.

A shows earlier form of apparatus In this S is stand from which rat jumps, L and R indicate location of holes in screen, M a metal apron to deflect rat through the opening if he jumps too high, FP is food platform, and N is net to catch animals that fall (From Lashley.[334]) B shows a later form of the apparatus. (From Lashley [335])

The stimulus-patterns are mounted on pieces of heavy cardboard 6 inches square, which are used to close the openings L and R in the screen. These cards are held in place at the lower edge by insertion in a groove in the platform (F.P.) just behind the holes, and at upper edge by a light spring, or rigid turn-button. The card which is to be used as the negative stimulus-pattern is fixed rigidly by means of the turn-button, so that when the rat jumps against it he will fall into the net; the card used to provide the positive stimulus-pattern is held only by the light spring, and falls back readily if the animal jumps against it. The rat, accordingly, lands on the platform and may obtain food. Another procedure that can be used to hold the cards against the back of the screen is to mount them on small wooden blocks. These permit the card to fall back easily when struck by the body of the jumping rat. To provide rigidity in the card bearing the negative stimulus-pattern, the upper end may be held rigidly by turn-button, and the block at lower edge is made to fit into a shallow groove in the platform.

(2) **Motivating Factors.**

(a) *Drive:* Hunger.

(b) *Reward:* If rat jumps against positive stimulus-pattern, it knocks over card, lands on platform and obtains food.

(c) *Punishment:* If rat jumps against negative stimulus-pattern, it collides with rigidly held card and falls into net.

(3) **Training Procedure.**

(a) *Preliminary training period.* The stand is placed close enough to the screen to permit rats to step through holes to the platform. They are allowed to explore and become familiar with the platform during the experimental period over an interval of 2 or 3 days. For an additional period of a few days food is provided on the platform. Then the stand is gradually withdrawn until after 10 or 15 trials, a distance of 25 cm. has been reached. As the stand is withdrawn, the rats learn to jump across the space to the platform. During this preliminary training period, no cards are placed against the openings. Some rats seem to show a disinclination to jump from the stand to the platform. To induce the act of jumping, it is found helpful to pinch the rat's tail or to push the rat off the stand. The stand may also be covered with an electric grill, and a shock be used to induce the rat to jump, though this procedure has been criticized by Munn[433] as introducing complications in the discrimination-phenomena. Maier found that a stream of air blown against the back of the rat was effective in getting it to jump.[380]

(b) *Training period.* Cards are now placed in the openings and the training in discrimination is begun. The position of positive and negative stimulus-cards is varied in chance order, in the same manner as for the Yerkes discrimination box.

(c) *Criterion of mastery of discrimination:* A sequence of 20 or 30 errorless trials.[334]

(d) *Trials per day:* 10 trials per day is representative.[335, 380]

(e) *Trials required for mastery:* The following figures are indicative of practice required prior to 30 or more errorless trials: Discrimination of black from white, average of 4.3 trials; of horizontal from vertical lines, 27 trials; of triangle with apex up from one with apex down, 28.6 trials.[334]

(4) **Control of Possible Accessory Cues to Discrimination.** Procedures used to prevent accessory cues to discrimination may be found in Lashley.[334] The cues considered are light from behind screen, extraneous cues from cards, tactile and olfactory cues, and cues from the behavior of the experimenter.

(5) **Tests of Validity of Method.** Tests of the validity of the method consist of the procedures used to indicate that visual patterns determine the discriminations and not unknown, uncontrolled factors. A variety of such tests were reported by both Lashley,[334] and Munn,[430] who subsequently tested the method.

(6) **Evaluation of Method.** Munn, while emphasizing the value of the method for certain purposes, contends that it has the following limitations: (a) Apparatus is adapted only to small animals which can be trained to jump; (b) Even for rats, a relatively long time is required to train animals to jump and (c) Even after rats have been trained to jump through the holes in the screen to the platform, they may frequently refuse to jump after having made a few mistakes and fallen into net.[433]

(7) **Illustrative Investigations:** Lashley,[335, 338] Krechevsky,[362] Maier.[380]

(8) **Modified Forms of Apparatus:** Lashley later described a somewhat modified form of his apparatus,[668] illustrated in Figure 117 B. Fields has described a multiple stimulus apparatus in which five stimuli may be presented simultaneously instead of two,[169] and Wolfle used a 5-window apparatus in which bridges were used to connect the choice platform with the stimulus windows. The rats were trained to cross the bridges instead of to jump a gap.[662]

Munn Discrimination Apparatus[431]

In attempting to determine why the Lashley technic made it possible for rats to learn to make a discrimination in 20 to 60 trials which they could not make in the Yerkes apparatus in 1000 trials, Munn was led to devise still another type of discrimination-box. He reports that it yields results comparable to the Lashley technic in the speed with which discriminations may be learned, and has in addition a number of other advantages.

(1) **Apparatus.** A diagram of the ground plan of the apparatus is shown in Figure 118.

Munn points out that the plan of the apparatus is similar, in many respects, to that of Fields' apparatus, and that the manner of presenting the stimuli is similar to that used by Lashley in the jumping-technic.

(2) **Motivating Factors.**

(a) *Drive.* Hunger. (During the training series described by Munn, rats are allowed to go for 2 days without food.)

(b) *Reward.* Food obtained by rat in food-box, after he pushes against door bearing correct stimulus-card.

(c) *Punishment.* Animal is given a shock as soon as it touches door with incorrect stimulus-card. Moment of shock is controlled by hand-key manipulated by experimenter.

(3) **Training Procedure.**

(a) *Preliminary period.* The rat is released from box A and is allowed to explore apparatus with doors open and food in food-box (4 or 5 trials). Then, at successive intervals, the following changes are introduced: one door closed (about 3 trials), both doors closed, but unlocked (about 4 trials), one door locked (6 to 10 trials). The preliminary habituation thus requires from 17 to 22 trials.

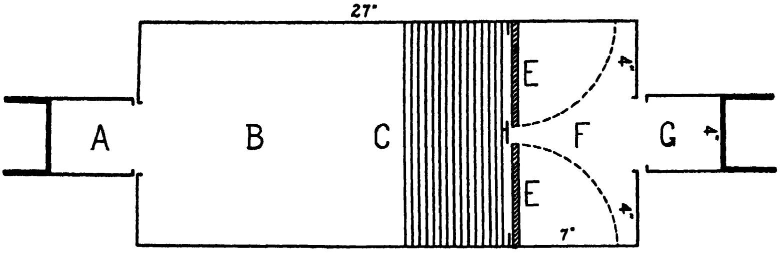

Fig. 118. Ground Plan of Munn Discrimination Apparatus.

A, entrance, B, discrimination chamber, C, electric grill, E, doors in which stimuli to be discriminated appear The doors open toward the rear of apparatus as pictured by dotted lines (From Munn [431])

(b) *Training series.* Cards to be discriminated are inserted in the doors, and animal obtains food if he pushes against positive stimulus-card, and receives a shock as soon as it touches negative card.

(c) *Criterion of mastery of discrimination-task:* Accuracy of 80% or better in a sequence of 20 trials.

(d) *Trials per training period:* 20 to 40 trials given daily.

(e) *Trials required for mastery:* The number of trials required for mastery varies with the nature of pattern. For discrimination between black and white cards, between 40 and 80 trials were required for different rats in a group of 7 containing 2 white and 5 hooded rats. The two white rats mastered the discrimination in 40 trials. (For data concerning other patterns, see Munn.)[431]

(4) **Control of Accessory Cues:** See Munn.[431]

(5) **Tests Made to Determine Whether Accessory Cues Were Operative:** See Munn.[431]

(6) **Evaluation of Method:** According to Munn, the advantages of this apparatus over that of Lashley are that it requires less preliminary training than Lashley's, and is adapted to a wider variety of animal forms.

(7) **Illustrative Studies:** Slater and Munn.[530]

(8) **Modifications:** Brown and Ghiselli[63] describe a multiple unit apparatus involving 12 successive discrimination units similar to that used by Munn. The special merit of the apparatus is its high reliability (reliability coefficent of 0.98), thus making possible comparisons of individual animals.

Morgan's Automatic Discrimination Apparatus[413]

Morgan has recently described a discrimination apparatus for rats which is said to provide an increase in efficiency over previous technics through the incorporation of a number of automatic devices. The apparatus combines features present in methods previously described by Skinner and by Smith. Smith's method was designed primarily for use with cats.[545] Skinner's method, which was designed for rats, will be described later in relation to conditioned-response technics. Morgan's apparatus may still be classed as a discrimination-method since both stimulus-objects are presented simultaneously.

(1) **Apparatus:** A longitudinal cross-section of Morgan's apparatus is shown in Figure 119. The animal is placed in compartment A. The two visual stimulus-objects to be discriminated appear at the two sides just above the levers, L. If the lever beneath the positive stimulus-object is pressed down, food is released into the tray T; if the other lever is pressed, the animal receives an electric shock. The two poles of the shock circuit are provided by the lever and a metal plate upon which the animal stands. Records of the actuation of the two levers are made by means of an ink-writing arrangement connected to the discrimination box. Control of stimulus-changes, and reward and punishment may likewise be made completely automatic. Details may be found in Morgan's papers.[413, 223]

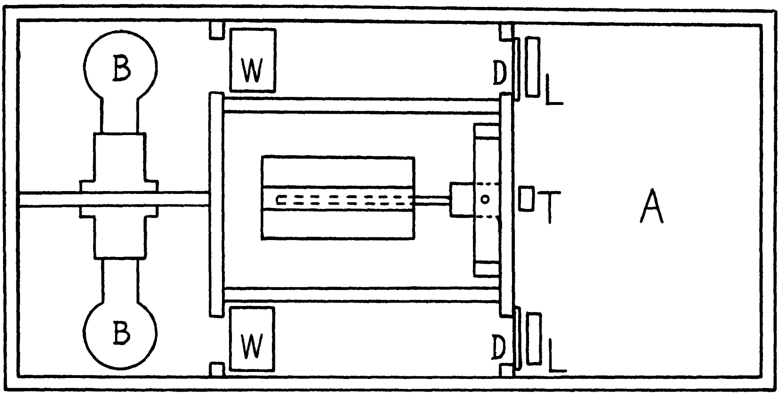

Fig. 119. Ground Plan of Morgan Discrimination Apparatus.

A, discrimination chamber, L, levers, T, food tray, B, light sources, W, water filters, D, diffusing panels (From Morgan [413]) (By permission of The Williams and Wilkins Co , publishers of The Journal of Comparative Psychology.)

(2) **Motivating Factors.**
  (a) *Drive:* Hunger.
  (b) *Reward:* Food (in powdered form) released in food-tray.
  (c) *Punishment:* Electric shock when wrong lever is pressed.

(3) **Training Procedure.**
  (a) *Preliminary period.* Animal is trained for 3 or 4 days in a Skinner type conditioning apparatus to push the lever to obtain food. It is then placed in discrimination-box and allowed to secure food by pushing either lever until 50 responses are made.
  (b) *Training period.* Positive and negative stimulus-objects are exposed. Shock first introduced after 50 to 100 such trials.
  (c) *Criterion of mastery of discrimination-task:* 75% correct responses for 30 minute test-period.[412]
  (d) *Trials per training session:* 100 trials. These require about 30 minutes.
  (e) *Trials required for mastery:* 200 to about 300 trials for discrimination of bright panel from dark.[413]

Special Types of Discrimination Apparatus

A wide variety of special forms of discrimination-apparatus (many of them based on the plan of the Yerkes box) have been developed to test the ability of the rat to discriminate different specific properties of stimulus-objects. A classification of various sensory and perceptive phenomena which have been investigated is given below. In the list of references associated with each topic, those of special interest, from the point of view of apparatus or technic, are followed by an asterisk. Others given represent chiefly investigations of factors on which the discriminative capacity in question depends. References to studies of the neurological mechanism involved, which have attracted a considerable amount of interest, are thus given in relation to the various special discriminative capacities.

Audition[433, 245*, 332, 58, 242, 243, 331, 452]
Somesthetic senses[433, 66*, 499, 538, 395, 1, 2, 361, 543]
Smell[433, 569*, 570]
Taste[433, 639, 483]
Vision
  (1) Brightness discrimination[433, 662, 120, 321, 461, 195, 337, 201, 603, 233, 185, 184, 518]
  (2) Hue discrimination[433, 432, 616, 435, 618, 619, 110]
  (3) Movement discrimination[433, 229]
  (4) Pattern discrimination[433, 63*, 167, 344, 338, 327, 339, 568, 231, 342, 103, 104, 315, 65, 345]
  (5) Size discrimination[433, 668, 522, 60, 106]
  (6) Spatial discrimination[433, 61*, 511*, 344, 605, 262, 573, 42, 182]
  (7) Temporal discrimination[433, 330, 95]
  (8) Visual acuity[433, 338]

Supplementary Technics

Series, based on chance-selections, for use in discrimination experiments may be found in a paper by Gellerman.[178] These series have been adopted as guides to the order of presentation of stimuli by a number of other investigators.[338, 413]

For a comparison of various technics of scoring the results of discrimination studies, see the report of Ghiselli.[183]

(*b.*) *Conditioned Response Method*

Although the conditioned response method has been one of the chief methods employed in tests of discriminative capacities of a number of other animal-forms, it has not been used extensively for this purpose with the rat. It is only in comparatively recent years that methods for the establishment of conditioned responses in the rat have been developed. Munn, for example, states in his monograph, which appeared in 1933, that the conditioned reflex method had not been used with rats up to that time.[433] A number of reports have, however, since appeared in which technics for eliciting conditioned responses in the rat are described. Reports on conditioning in the rat have been largely concerned with the phenomena of learning. Our survey of the available technics will therefore be given in the section on learning. These technics may be applied to the investigation of discriminative capacities if factors of learning are controlled. Reports of the explicit application of conditioning procedures or principles to the testing of discriminative capacities have been made by Skinner,[532, 533, 534, 535] Stott and Ruch,[567] Hunter,[295] Hull,[270] and Hanford and Morgan.[223]

EMOTIONAL BEHAVIOR

Any discussion of the topic of emotion is handicapped by the difficulty of stating with precision, and yet with sufficient completeness, the characteristics of behavior which are to be regarded as emotional. The widespread character of the bodily response interferes with the formulation of distinctive criteria. This difficulty is the more pronounced in the field of animal psychology, in which verbal reports by the reacting individual are not available as guides. Judgments of the presence or absence of emotion must be based on externally observable changes in the animal, a requirement which has been more difficult to meet satisfactorily in the field of emotion than it has in other behavior-fields. Despite this difficulty, a number of reports have been made in recent years of the investigation of emotional behavior in the rat. The technics involved may be summarized in relation to the following three topics: (1) Emotion-exciting situations; (2) Measures of degree of emotion; and (3) Conditions or phenomena associated with emotion.

Emotion-Exciting Situations. A variety of situations for exciting emotional behavior in the rat have been used by different investigators. A possible classification of these situations is as follows:

(1) Novel situations, e.g., the open field situation of Hall, and of Anderson;[208, 11, 446, 13] (2) exposure to disturbing stimuli, such as bright illumination,[446] a loud buzzer tone,[446] a cat,[123, 613] intermittent shocks by way of floor of living cage,[443] tilting of glass floor of cage to upset balance and produce sliding;[446] (3) situations requiring possibly disturbing responses, such as escape from living cage;[11] emergence from dark tunnel to open food-box,[11] swimming or wading through water;[11, 13, 446] and (4) arrangements imposing restraints on free movements of the animal.[164, 446] Other technics which should probably be included in the present list are certain of those employed to elicit neurotic behavior. Many of them are similar to the situations mentioned in the list above.

Measures of Degree of Emotional Response. To provide a measure of the magnitude of an emotional response, three general types of index have been proposed: (1) the score obtained by a rating scale procedure involving different types of response;[563a] (2) the amount of excretion (defecation or urination) exhibited by the animal during a standard interval in the test situation;[208, 11, 446] and (3) the length of time required by the animal to perform certain disturbing tasks.[11]

A description of Stone's rating-scale procedure may be found in Munn.[433] In the use of the second type of procedure mentioned above, the amount of defecation or urination has been scored in different ways. Thus Hall[208] finds it a satisfactory procedure to count the number of *trials* in which a rat defecates or urinates when placed in a novel situation under standard conditions. The situation is provided by a circular space, 8 feet in diameter, surrounded by a wall 2 feet high. The rat is placed in this enclosure for a standard test-interval (e.g., 2 minutes) and one trial given a day for a fixed number of days. Other scoring procedures consist in counting the *percentage* *of animals* in a group which defecated or urinated under given experimental conditions,[208] or in counting the number of *fecal boluses* excreted by a given animal in a standard test interval.[208, 11]

To supplement the defecation test, Anderson used as an index of emotional behavior the time required for a rat to perform the first two tasks specified under (3) in the preceding section.

Another type of index which could probably be used to advantage is one based on the relative lymphocytosis reported by Farris[164] to occur in the rat as a result of emotional excitement. Physical restraint of movements provided the emotion-exciting situation. Other characteristic signs of emotion, such as defecation and urination were also present. The blood samples used in making the blood-cell counts were obtained readily by clipping off the end of the tail with a sharp razor blade.

Relation of Emotional Behavior to Other Phenomena. The situations and tests described in the preceding sections have been used in determining the relation of emotional behavior to a number of biological and psychological phenomena.

Biological phenomena:
(1) Blood-cell counts[164]
(2) Endocrine conditions[14, 212]
(3) Hereditary factors[212]
(4) Neural factors[47]
Psychological phenomena:
(1) Activity[210]
(2) Drives and needs[208, 209, 11, 12, 14]
(3) Social conditions[13]
(4) Variability of behavior[163, 211]

LEARNING

Under the topic of learning, we are concerned with the processes by means of which an animal becomes able to adjust to new situations, situations for which its previous repertoire of habits is not adequate. Learning may, accordingly, be defined as the modification of the adjustive capacities of the animal as a result of behavioral activity.

In the analysis of the processes by means of which an animal learns to adjust to new situations, it has been found useful to mark off two separate stages: (1) the stage of initial adjustment or achievement; and (2) the stage of fixation or stabilization. Thus, when an animal is placed in a new situation, and is impelled by an internal drive of some sort to persistent activity in the direction of a particular goal, the first stage of learning may be regarded as completed when the animal first succeeds in reaching the incentive. If, now, the animal is again placed in the initial situation, and the same drive is operative, a new process of adjustment begins, which may again end with the reaching of the incentive. In successive trials of this sort, the behavior exhibited by the animal in reaching the incentive is frequently found to be modified progressively in such a way that the animal is finally able to attain the incentive in the most direct way possible, without the many unnecessary acts invariably observed in the initial trial. The latter series of changes in the adjustment process involves a process of reorganization and stabilization of the animal's adjustive behavior, and implies corresponding changes in its internal adjustive capacities. For convenience, we may refer to it as the stage of fixation. This term should not be taken to imply, however, that during this second stage of learning, there is no change in the pattern of behavior of the animal in successive trials. On the contrary, the behavior is likely to exhibit a continued course of modification and reorganization.

Although the two stages of learning just described may be regarded as present in every complete process of learning, it has not been customary to deal with them separately in every investigation of the course of learning in different types of situation. While some types of experimental learning situation have been found well adapted to investigation of both stages of the learning process, others have been found better suited to the investigation of one or the other stage.

The *problems* for which different technics have been devised in research upon learning processes in the rat have been of two general kinds: first, the problem of determining the course or pattern of behavior exhibited during any given stage of learning, and, second, that of determining the specific conditions or factors on which the course of learning and salient end-points in its course depend.

The chief special requirements in the development of technics for the investigation of such problems in the rat have accordingly been: (1) the provision of an adequate variety of controlled situations which might serve as new situations for the rat and in which its behavior during either of the stages of learning might be observed; and (2) the devising of technics for controlling and measuring all the conditions which might influence the learning process. A further requirement common to all learning investigations has been the need for motivating the experimental animal so that it will display persistent behavior in the direction of some incentive. The hunger drive has been found the most convenient for this purpose but it is, in principle, feasible to utilize other drives as well, and these have occasionally been used. The nature of different types of drives which might be employed is considered in the section on motivation.

The basic methods used for investigation of learning in the rat may be classified in terms of the type of situation in which the animal is placed and in which the readjustment designated as learning occurs. Four methods are conventionally differentiated: (1) the conditioned-response method; (2) the discrimination-response method; (3) the maze-method; and, (4) the problem method. After each of these methods has been considered, a final section on "common factors in learning" will deal with studies not limited to the use of any one of the four special methods.

(*a.*) *The Conditioned-Response Method*

The conditioned-response method, like the discrimination-response method, is a technic which brings about a new association between stimulus and response. A situation which is at first incapable of consistently eliciting a given response on the part of the animal becomes capable of doing so as a result of certain training procedures. The stimulus and response may now be said to be associated and we speak of the stimulus thus associated with a particular response as the positive stimulus. The term association is used here only in a factual sense, and does not imply any particular explanatory mechanism. The fact that such new associations are formed as a result of both the discrimination-response method and the conditioned-response method is the reason both of these methods may be regarded as suitable for investigation of the phenomena of learning, since the outstanding phenomenon in learning may be said to be the formation of new associations. Whether the manner in which such associations are formed in the two methods just mentioned is characteristic of all learning is a question about which there is still controversy.

In the discrimination-response method, the training procedure used for establishing new associations involves rewarding the animal with food when it moves in the direction of the positive stimulus, and punishing him with an electric shock when it moves in the direction of the negative stimulus. As a result, the movement of the rat in the direction of the positive stimulus finally becomes associated with that stimulus.

In the case of the conditioned-response method, the procedure followed can be regarded as fundamentally the same. The purpose of the experimenter is, here too, to bring about an association between a particular stimulus or property of the stimulus-situation and a particular response. The positive stimulus in this case is called the conditioned stimulus, and the positive response, the conditioned response. In one form of conditioning procedure, the animal is first led to make the response desired by having presented to it some stimulus that is already capable of bringing about the given response. This stimulus is called the unconditioned stimulus. If now, at the same time (or a short time before) the unconditioned stimulus is presented, the conditioning stimulus is also presented, the animal will naturally continue to make the appropriate response to the unconditioned stimulus. If this procedure is repeated a sufficient number of times, it is found that the conditioning stimulus, even though presented alone, becomes capable of eliciting the same response. The conditioned response and conditioned stimulus have become associated, and the animal is said to be conditioned to the new stimulus.*

In the traditional studies of conditioned response in the dog, two principal types of response have been used: (1) Salivation by the animal, such as may be brought about in the hungry animal by the sight of food; and (2) the withdrawal of a leg, such as is brought about by means of an electric shock serving as the unconditioned stimulus. In the case of the rat, somewhat different types of response have been utilized. The investigations which so far have been made of conditioned responses in the rat may be classified under four headings: (1) the development of procedures for establishing conditioned responses; (2) the investigation of the traditional phenomena of conditioning—such as extinction, reinforcement, satiation, and the like; (3) determination of factors on which the phenomena of conditioning depends; and (4) investigations or discussions of the part played by conditioning phenomena in various forms of learning or behavior. Our treatment of the last three topics will be limited to a classification of the relevant literature. The procedures developed for establishing conditioned responses in the rat will be considered in some detail.

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* See Skinner (537, p. 19ff) for a distinction between two types of conditioned reflex.

Establishing Conditioned Responses

Lever-operation Technics. Skinner has described a method by means of which the rat might be trained to respond to a given stimulus by the operation of a lever. The apparatus used consists of a chamber in which is placed the animal used as subject. Located in one of the walls of the chamber is a lever, which, when pressed down by the rat, may lead to the release of a standard pellet of food into a tray underneath. Other devices may be incorporated to provide for presentation of stimuli to the animal. Associated with the apparatus is an automatic recording arrangement which permits a temporal record to be kept of the operation of the lever and of the time of presentation of stimuli. The significant features of the animal's behavior during the training period may thus be recorded without the experimenter's presence and continued observation.

In this situation, the hunger drive provides the motivation. If the animal responds to the positive stimulus by actuation of the lever, it is rewarded by a food pellet of standard size and shape. If it makes this response under any other circumstances, the food pellet is not released. The provision of the food pellet is spoken of, in accordance with conventional conditioning terminology, as a reinforcement of the lever-depressing response. Through regulation by the experimenter of the responses which are reinforced, the animal may be readily trained to discriminate between two different stimuli. For technical details concerning the method and its use, Skinner's publications should be consulted.[537]

Somewhat modified types of apparatus making use of a lever-device for conditioning the animal have been described by Youtz[675] and by Hanford and Morgan.[223] Through the introduction of two such lever-arrangements in the same apparatus, Morgan developed a discrimination apparatus for rats which has been already described in the section dealing with the discrimination-response method. Finan[172] has very recently described a modification of Skinner's technic in which the release of food into the food-tray is brought about by the interruption of a light beam shining on a photo-electric cell, instead of by the depression of the lever. The positive response of the animal now becomes its movement to a particular part of the experimental chamber rather than the depression of the lever.

Heron and Skinner[251] have described an ingenious apparatus in which 24 experimental chambers of the Skinner type may be operated simultaneously. The recording apparatus permits the immediate registration of curves which show the rate of response of the individual animals, and also mean curves for the total group or for sub-groups of animals. The authors call attention to the suitability of the apparatus for investigation of a wide variety of problems, principally in the fields of learning and motivation, and to the great saving of time effected through the provision of mean curves for groups of animals.

Technics Involving Localized Reflexes. Schlosberg,[515, 516] Kappauf and Schlosberg,[309] and Hughes and Schlosberg[269] have attempted to develop technics for conditioning in the rat which are modelled rather closely on the classical procedures. The animal is restrained in a holder so that free locomotor response is not possible.

The responses which Schlosberg tried initially to condition were withdrawal of tail or leg from a grill, with shock to these parts being used as the unconditioned stimulus. Schlosberg found that the response to shock also included rather diffuse responses such as changes in breathing rhythm, sharp inspirations, and squealing. These, he found, could be quite readily conditioned to a buzzer-signal or to light, whereas localized tail or leg withdrawal were more refractory to conditioning. In a final study with Hughes, evidence was presented to show that a sharply localized response, the lid reflex to a puff of air against the cornea, could be readily conditioned even though the animal was restrained in a holder, as in the earlier attempts.[269]

Another instance of a conditioning technic which belongs in this section is that of Cook.[114] In attempting to establish an experimental neurosis in the rat, he first established a conditioned flexion response of the right foreleg to a visual stimulus. The animal was strapped in a holder apparently similar to that used by Schlosberg, and the unconditioned response elicited by an electrical shock.

Technics Involving Escape-Response of Total Animal. A number of investigators have described technics for establishing conditioned responses on the basis of an escape response of the animal as a whole from an electrically charged grill. The first apparatus of this sort seems to have been described by Warner,[629, 630] although it was not designed or represented as an apparatus for establishing conditioned responses. It consisted of a box with two compartments separated by a partition. On the floor of each was a grill which could be charged electrically. When the grill on which the animal rested was charged, the rat could escape by jumping over the partition to the other end of the box. If now the grill in the second compartment was charged, the animal could escape by jumping back again to the original compartment. If a given stimulus, such as the sound of a buzzer, was presented just before the charging of the grill for a series of trials, the animal became conditioned to the stimulus, and would respond by jumping to the opposite end of the box before it received a shock from the grill.

The use of an escape-type of response for establishing conditioned responses has been reported subsequently by a number of other investigators. Elliot and Treat,[157] Gentry,[181] and Cook[114] report the use of technics very similar to that of Warner, in that the response of the animal consists in movement to the opposite end of the training-box. In Cook's apparatus, a space four inches wide was located between the two ends of the box in place of the partition. A permanently charged grill at the bottom of this space prevents the rat from escaping to this position. Elliot and Treat do not furnish details concerning the construction of their apparatus.

Hunter[288, 286] made a further modification of the technic by building an apparatus that permitted the animal to escape in either direction from the grill on which it was resting. The apparatus was roughly circular in shape with eight compartments located around its circumference. At the bottom of each compartment was a grill which could be electrically charged. The rat could escape by running to the next compartment in either direction. Doorways were used to separate the compartments instead of partitions, since Hunter, in a trial of the Warner apparatus, had found that the rats would sometimes escape by sitting on the partition. Hunter's apparatus makes it unnecessary for the rat to turn in order to get to an unelectrified compartment.

A second type of modification of the Warner technic has been described by Hunter and Pennington,[295] and by Stott and Ruch.[567] In this type of apparatus, one possible resting position of the animal is the floor of the cage, which consists of a grill which can be electrically charged. The other possible position is a platform located above the floor, also covered with a grill. When one grill is charged, the animal can escape by jumping up (or down) to the other grill. Hunter and Pennington devised their apparatus for testing the rat's tone-discriminative capacities; Stott and Ruch to test the animal's time-discriminative capacities. The apparatus of the latter authors is somewhat more complicated in that it also includes devices for automatic recording of the animal's location, and for dropping the rat back to the bottom of the cage from the platform after standard intervals of time.

Phenomena of Conditioning

Investigation of various phenomena of conditioning, such as the rate of establishment of conditioned responses, extinction, spontaneous recovery, and disinhibition have been reported in papers by Hunter,[286, 288, 289, 290, 291, 292] Prosser and Hunter,[465] Skinner,[537] Youtz,[673, 674] Ellson,[159] and Brown.[68]

Factors Influencing Conditioning

Investigation of the dependence of phenomena of conditioning on various factors have been reported by Ingebritsen,[310] Elliot and Treat,[157] Porter,[462] Voitonis,[612, 613] Ellson,[160] Sackett,[513] Youtz,[675] Keller,[310] and Finan.[173]

The Rôle of Conditioning in Learning Performance

A number of papers have appeared covering the rôle which conditioning phenomena may play in learning performances that are not obvious instances of conditioning effects. Some of these papers attempt to show that the performance in question may be explained by conditioning principles;[678, 673, 674, 275, 274, 471] others take the opposite point of view.[578, 581]

(*b.*) *The Discrimination-Response Method*

The nature of the discrimination-response method has already been described in relation to technics for investigating discriminative capacities in the rat. In its use for that purpose, the focus of interest of the experimenter lies not in the learning procedures as such, but in the discrimination which the animal is called upon to perform. The learning which goes on is merely a tool which permits the experimenter to determine whether the rat is capable of a given discrimination. The nature of the learning phenomena involved in the discrimination-response method may, however, be considered in their own right, and the method used for the investigation of learning, with the discriminative capacity involved being shunted to the rôle of constant.

Expressed in more formal terms, the achievement of the rat in showing the ability to discriminate consistently (or in accordance with any given criterion) between two given stimuli may be said to be a function of two factors: (1) the capacity to make a given discrimination and (2) the capacity to learn, to make the appropriate association between a given stimulus-object (or the perception of it) and a particular response. If conditions are arranged so that the learning-capacities required may be assumed, or regarded as a constant in a given experiment, then the final successful performance may be taken as an index of the capacity for discrimination. The discriminative capacity may be treated as a variable and its relation to different factors investigated. This is the situation in the use of the discrimination-response method in investigation of discriminative capacities. On the other hand, if a given capacity to discriminate may be regarded as a constant, the capacity to learn to make the differential response required may be regarded as a variable and its dependence on the conditions of the experiment investigated. The fact that different discrimination-response technics have been developed which differ in the number of trials required for a given discrimination to be learned is itself indicative of the significance of learning factors in this type of experiment.

Following is a classification of investigations of learning involving the discrimination-response method. References to studies of discriminative capacities by means of this method are classified in an earlier section.

I. Characteristics of the learning process:[318, 579, 463, 379, 205, 326]
II. Discrimination-response learning as a function of the following factors:
    A. *Biological factors:*
        1. Endocrine secretions[563, 634]
        2. Neural factors[439, 440, 186]
    B. *Psychological factors:*
        1. Environmental background[499]
        2. Motivational factors[140, 424, 429, 425, 421, 422, 147, 398, 78]
        3. Previous learning (transfer, etc.)[401, 105, 232, 202, 308]
        4. Sensory factors[177, 188]
        5. Theoretically derived factors[396, 678, 108, 581, 203]

(*c.*) *The Maze Method*

The maze has been one of the most widely used devices for investigation of learning-processes in the rat. Mazes have been built in a wide variety of types, and extensive studies have been made of the suitability of different types for different kinds of research problems. A maze consists essentially in (1) an entrance-chamber in which the animal is placed at the beginning of a run, (2) a goal-chamber, usually a food-chamber, at a fixed spatial locus, in which is contained the incentive for the animal's activity, and (3) the run-ways, or passage ways that the animal may use in running from the entrance-box to the goal-box. These run-ways are of two kinds, connecting run-ways, which lead the animal closer to the goal, and blind alleys, which terminate in an obstruction. The problem which is usually set for the animal by the experimenter is that of getting from the entrance-box to the goal-box by the quickest and most direct route.

Types of Mazes. Mazes may vary from each other in three possible directions: spatial-temporal character, structure of run-ways, and specific pattern. In terms of the first variable, mazes may be either of the *spatial* or *temporal* type. In the spatial maze, a set of pathways are arranged so as to form a fixed spatial pattern. In a maze of this type special sensory cues associated with each unit of the maze may guide the animal into the correct pathways. Illustrations of spatial mazes are given in Figures 120, 121, and 122.

In the temporal maze,[281] the rat's route to the goal is not determined by a fixed spatial pattern. The physical structure of the apparatus is relatively simple. It may be characterized as essentially a rectangle with a *central* cross-bar, in addition to the ends, joining the two long sides of the rectangle. The animal starts down the central path provided by the cross-bar. At the end, it may turn to the left or the right. After taking the path to the right or to the left, the animal returns to the central pathway. In successive cycles of this sort, the particular side path left open may be varied according to the plan of the experimenter. The other path is blocked. The animal, in order to eventually reach the food box, may be required to follow any desired sequence of turns. Thus, in the single alternation pattern, the sequence followed may be right, left, right, left, for the selected number of cycles. In the double alternation pattern, the sequence may be "llrrllrr" and so on. The turn to be made at any time depends on the previous sequence of turns and not on the identity of the specific run-way being traversed. Such temporal mazes have been of value in the analysis of sensory factors which guide maze behavior, and in investigations of symbolic processes in the rat.[285, 281, 294] Evans[162] has described a temporal water maze, with which he investigated the way in which the rat learns to solve the simple alternation problem. The use of water for the maze was adopted as a means of keeping the rat active during any trial without feeding it during the trial.

A second differentiating feature in mazes is the structure of the run-ways. They may be either of the alley or elevated type. In the alley maze, the animal is kept from leaving the maze pathways by side-walls perpendicular to the floor. The animal must run in the alleys thus formed. An alley maze is illustrated in Figure 120. In the elevated maze, such side-walls are absent. The rat is prevented from leaving the maze through the elevation of the surface constituting the pathway to a height sufficient to keep the rat from jumping. An illustration of a block elevated maze is given in Figure 121.[128]

In other forms of elevated maze, the run-ways are supported by a skeleton-framework.[404, 346]

Finally, mazes may vary from each other in the specific nature of the pattern used. According to a survey by Warden and Warner[631] over a hundred different maze-patterns have been used since the maze was devised for animal studies. Among the most frequently used of the early types have been the Watson circular maze[637] and the Carr square maze.[631] In recent years, these have been discarded for more reliable mazes, examples of which are the multiple-T maze devised by Tolman, Tryon, and Nyswander,[583, 585] and the multiple-Y maze devised by Warner and Warden.[631] Illustrations of these mazes are shown in Figures 120 and 122. Among temporal mazes, the pattern is provided by the particular sequences of right and left turns required of the animal before it is permitted to reach the goal. Thus possible patterns are single alternation (rlrlrl . . . and lrlrlr . . .) and double alternation (rrllrrllrr . . . and llrrllrrll . . .) sequences.

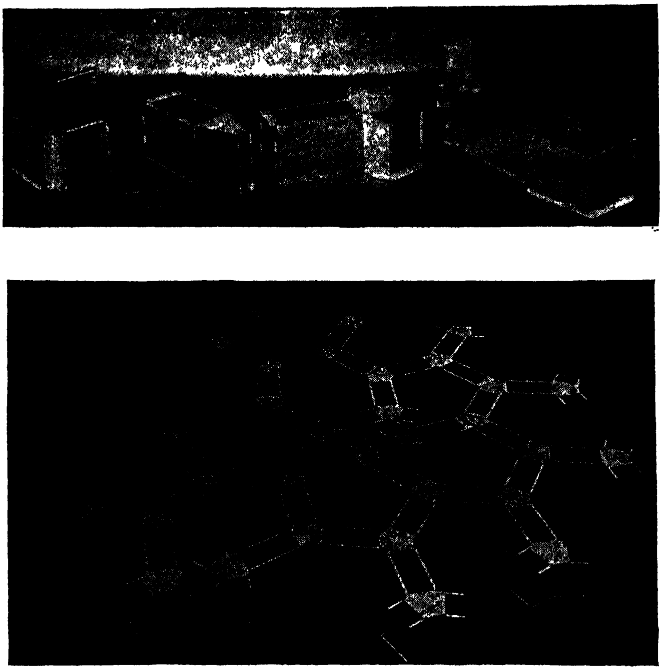

Fig. 120. Warner-Warden Multiple-Y Maze.

Upper cut shows the four types of unit of which the maze is constructed, from left to right, entrance box, pathway unit, cul-de-sac, goal-box. Lower cut shows one of the possible patterns—an arrangement including 15 culs-de-sac (From Warden,[628] illustration courtesy of Geo. H. Wahmann Mfg Co., Baltimore)

Reliability. In 1901, Small[542] first reported the use of the maze in the investigation of learning in the rat. Despite a considerable number of maze-studies by subsequent investigators, it was not until 1917 that the importance of maze-reliability was stressed. In that year Patterson[447] reported the results of an examination of the reliability of the Johns Hopkins circular mazes. Since that time, numerous investigations of the reliability of different forms of maze have been reported.

Procedures for Determining Reliability. The specification of the reliability of any maze measure represents a specification of the extent to which it is independent of chance factors, and is measured by the consistency with which a given score (or set of scores) is obtained in repeated measures of the same individuals. Theoretically, the reliability of any measuring device is most directly represented by the probable error (or standard error) of a single measure.[328] In practice, however, it has been conventional to obtain an index of the reliability of maze measures in terms of the reliability coefficient obtained from two sets of scores upon the same group of animals. The procedures that have been proposed for this purpose are the following: (1) correlation of scores on odd versus even *blinds*; (2) correlation of scores on different parts of the maze (e.g. first half versus second half); (3) correlation of scores on odd versus even *trials*; (4) correlation of scores on different series of trials (e.g. first ten versus second ten); (5) correlation of scores on an initial series of trials with those on a second comparable series performed after an interval of time has elapsed (test-retest method); (6) correlation of average time or error-score per trial with number of trials required for mastery; and (7) correlation of Vincent scores rather than raw scores. Leeper in his evaluation of the different methods is inclined to favor numbers 3, 4, and 5 in the above list, and points out more or less serious flaws in the others.[348] Other papers have been published and may be consulted for dis-

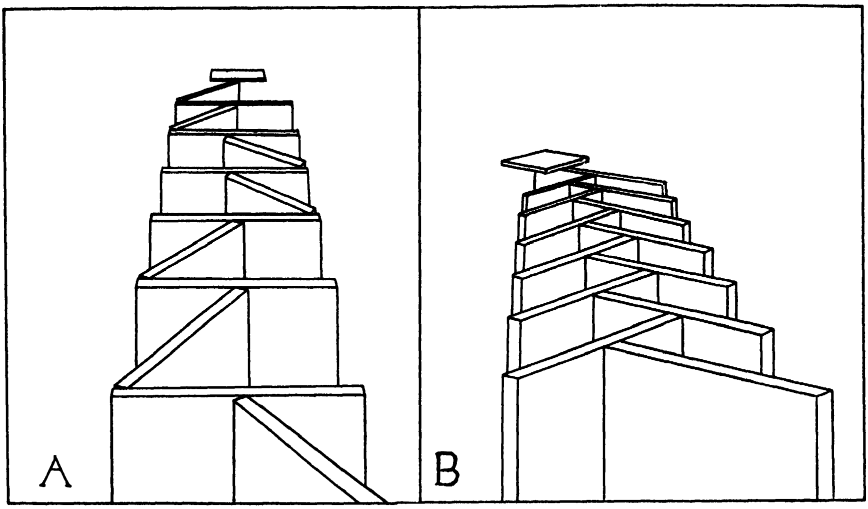

Fig. 121. A Block-Elevated Maze.

The two figures show two different arrangements of the units of the maze (From Dennis.[128]) (By permission of The Williams and Wilkins Co., publishers of The Journal of Comparative Psychology.)

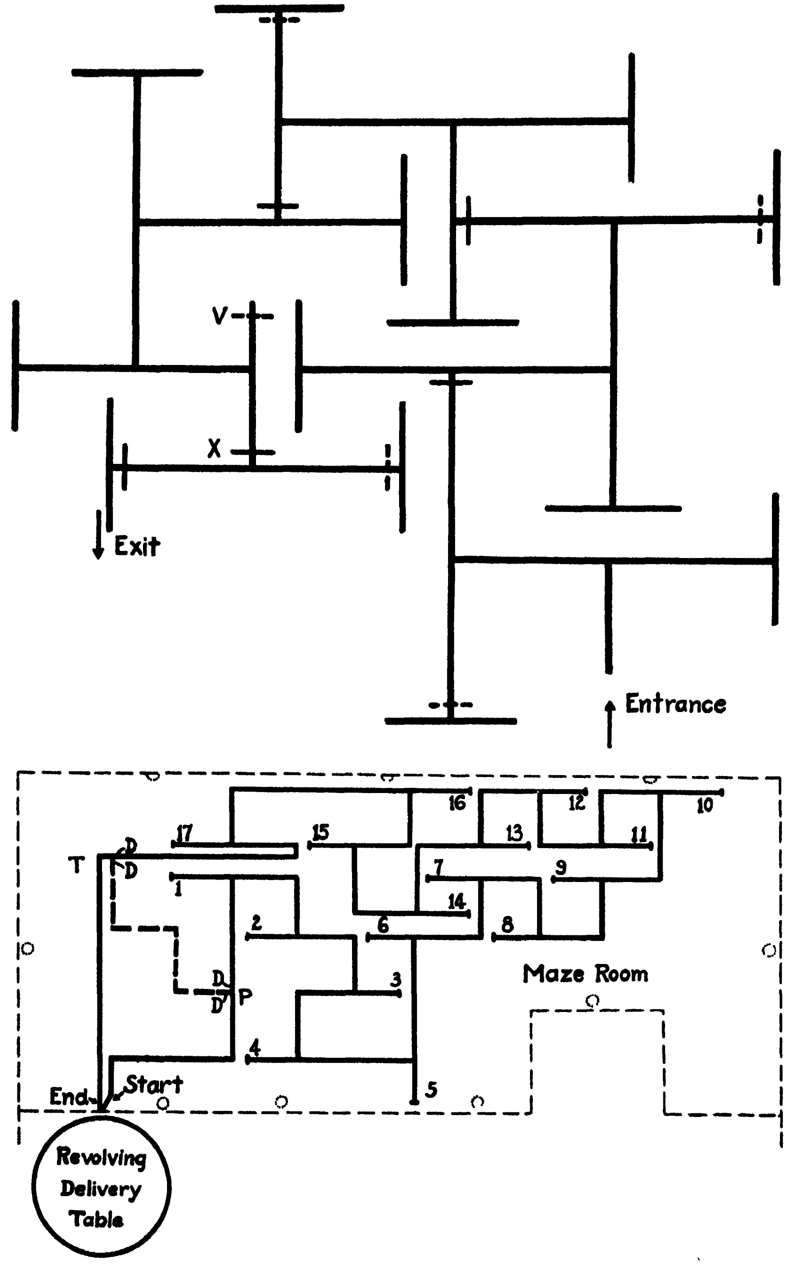

Fig. 122. Diagrams of Two Multiple-T Mazes.

A. Stone multiple-T maze Solid cross-lines at right angles to maze path represent retracing doors, dotted cross lines represent pseudo-doors to give appearance of real door (From Heron[246]), B. Tolman, Tryon, and Jeffres automatic recording maze Numerals indicate sequence of culs-de-sac. (From Tryon.[592]) (By permission of The Williams and Wilkins Co., publishers of The Journal of Comparative Psychology.)

cussions of methods for measuring maze reliability and other theoretical questions relating to reliability.[282, 296, 100, 283, 590, 591, 267, 333, 592, 584, 565, 246, 436, 293]

Reliability of Mazes. To aid in the selection of mazes Table XX has been constructed. It includes those mazes which have been found to have a reliability of 0.69 or greater. This coefficient of reliability was obtained by Hunter for the T-maze which he tested in 1924, and was the highest value reported for any maze up to that time. The mazes in the table are listed in the order in which the description of the maze was reported in the literature. In a number of instances, the test of the maze's reliability was not made until some years later. The last column of the table specifies the values obtained for the reliability coefficient for different mazes. The coefficients recorded are those obtained by correlating errors in odd versus even trials. The only exception is the reliability coefficient of 0.69 obtained for Hunter's simple T-maze; this coefficient was based on correlation of total *time* on odd versus even trials. Many of the reports from which the data of the table are drawn also specify the reliability-coefficients obtained by other procedures. In order to keep the table from becoming too cumbersome these values have not been included.

It will be noted that many of the reliability coefficients given have been corrected by means of the Brown-Spearman prophecy formula.[201a] This correction has the effect of raising the coefficient somewhat, and is intended to provide an estimate of the reliability of the whole series of trials; the raw correlation gives the reliability coefficient for only half the trials, since odd and even trials in the same learning series are being correlated. A number of investigators, notably Leeper,[348] and Newman,[436] have, however, questioned the applicability of the formula to maze learning data, on the grounds that the two sets of scores correlated are not independent.

Training Procedure. The specific procedure to be used in conducting maze experiments will naturally vary somewhat with the problem. Descriptions of possible procedures may therefore be found in the papers reporting particular types of investigations. Many of the reports on maze reliability cited in Table XX give detailed specifications of experimental procedure, and discuss their influence on the reliability of the maze. Warden, Jenkins, and Warner[628] and Jackson[303] call attention to the importance of a standard procedure in the preliminary habituation period, before training in the maze itself is begun.

Recording Technics. Three different procedures may be used for *recording* the relevant features of the rat's behavior during the running of the maze: (1) Direct observation of the animal's behavior and tabulation by hand of relevant features on data sheet; (2) graphical representation of the course followed by the animal through the maze; and (3) automatic recording of particular features of behavior. The first procedure has probably been used most frequently, on grounds of convenience and economy of preparation required. Special technics by means of which the experimenter may obtain a graphical record of the animal's course have been described by Watson[637] and by Yerkes and Kellogg.[664] Examples of investigations in which use was

TABLE XX

Data on spatial mazes of relatively high reliability

	Description of Maze			Tests of Reliability			
First Described by:	Type of Maze	Date Described	Ref in Bibliography	Tested by	Date	Ref in Bibliography	Reliability Coefficient for Errors (odd vs even trials)
Hunter	Simple T	1922	282	Hunter and Randolph	1924	296	.69[1] [time]
Warden	Mult-U (10 blinds)	1924	620	Husband	1929	297	.88 (c)[2]
Tolman, Tryon and Jeffres	Mult T 17 blinds self-recording (maze x)	1925 1929	283 585	Tryon	1930	592	.9876 (c)
Stone	Mult T[3] (14 blinds)	1927	584	Tolman and Nyswander	1927	584	.909 (c)
				Stone and Nyswander	1927	565	.59 (c) to .97 (c)[4]
				Heron	1930	246	similar to values obtained by Stone and Nyswander[5]
				Leeper	1932	348	.10 to .90
Warner and Warden	Mult Y	1927 1929	631 623 624	Jackson maze x maze y	1932	303	 .76 (c) .95 (c)
Miles	Mult T-elevated (15 blinds)	1927 1930	404 405	Miles Corey	1930 1930	405 116	.73 and .78 .89
Stone	modified Carr	1928	552	Stone	1928	552	.38 (c) to .87 (c)
Shirley	modified Hampton Court	1928 1929	526 333	Shirley	1928	526	.62 to .80
Yoshioka	alternative triang. or pentag. routes	1929	666	Yoshioka	1929	666	.789 (c) to .913 (c)
Tryon	Mult T 20 blinds hand-recording (maze y)	1930	592	Tryon	1930	592	.9682 (c)
Dennis	Simple U-shaped	1930	129	Dennis	1930	129	.90 or greater

TABLE XX (*Continued*)

Data on spatial mazes of relatively high reliability

	Description of Maze			Tests of Reliability			
First Described by:	Type of Maze	Date Described	Ref in Bibliography	Tested by	Date	Ref in Bibliography	Reliability Coefficient for Errors (odd vs. even trials)
Ligget	Multiple square unit (4 units)	1930	356	Ligget	1930	356	.82
Heron	Mult Y 12 units self-recording	1933	247	Rundquist and Heron	1935	510	.89 (rank order corr.)
Lathan and Field	Multiple unit, elevated	1936	346	Lathan and Field	1936	346	.94 (c)
Brown and Ghiselli	Mult T (18 units)	1938	64	Brown and Ghiselli	1938	64	.99 (c)

[1] *r* determined by correlation of total time on odd vs. even trials.

[2] (c) following coefficient means it has been corrected by the Brown-Spearman prophecy formula [201a]

[3] There were slight variations in the maze used in the tests of reliability by the four investigators listed The basic pattern was, however, the same in all. The maze tested by Tolman and Nyswander was the mirror image of the maze tested by the 3 later investigators. The only other difference in the successively tested mazes was in the number of retracing doors: 3 in the Tolman-Nyswander maze, 4 in the Stone-Nyswander maze, 5 in the Heron maze, and 1, 4 and 13 in different parts of Leeper's investigation. The reliability coefficient tended to increase with increase in the number of retracing doors

[4] In instances in which a range of values is given, the different values correspond to the use of different conditions in the experiments or different computation-procedures for determining the reliability coefficient.

[5] Heron reports that he found values approximating those obtained by Stone and Nyswander when he used their procedures of calculating reliability When, however, he used the test-retest method, he obtained values notably lower, namely, *r* values of 0 326, 0 376 and 0 805 for error-scores The different values were obtained for different experiments with different length intervals between test and retest. The value of 805 was obtained with the shortest interval used, 20 days Leeper, who also determined test-retest reliability coefficients found that his values were comparable with those he obtained by correlating odd with even trials, a result contrary to that of Heron Leeper suggests that the greatly reduced *r's* obtained by Heron for the test-retest method may represent a change in the subjects' abilities due to the long interval used between test and retest.

made of graphic representations of the pathway taken by the rat are those of Dennis[133] and of Dashiell.[124]

Automatic methods for recording the rat's maze behavior have been reported by Tolman, Tryon, and Jeffres[585] and by Heron.[247] Henry and Brown[244] describe an improved circuit for Heron's device, and Henry[241] describes automatic indicators and recorders of the times at which electric shock is administered. The merit of such automatic methods is that they permit a saving of time and effort on the part of the experimenter, and increase the objectivity of the records. Such objectivity is considered by Tryon to increase the reliability of maze-scores.

Scoring Technics. The variables in terms of which the rat in the maze may be scored include time per trial (total time, or active time—time spent in running), errors per trial, trials to reach a given norm of mastery, and distance traveled (total distance, or distance in excess of true path).

Discussions of the relative merits of different scoring procedures may be found in the papers of Tolman and Nyswander,[584] Stone and Nyswander,[565] Leeper,[348] Warden,[631, 622] and Ligget.[356a] Error scores are quite generally regarded as most satisfactory and have been frequently used. Three kinds of errors have been differentiated: forward entrances into culs de sac, retracing parts of true path, and retracing path into culs de sac. The first-named error is quite commonly regarded as of most significance. Retracing errors are, in fact, eliminated in certain mazes, in which retracing doors are used. Warden[622] and Leeper[348] have pointed out the lack of a standard criterion for labelling errors and the possibilities of confusion. This factor must be considered when results of different studies are being compared. This difficulty is reduced when an automatic recording maze is used.

Treatment of Data. The manner in which the data are treated will depend of course on the particular problem being investigated. Certain fairly standard procedures have, however, been developed as a preliminary to the plotting of learning curves, and these may be mentioned. A basic method consists in the plotting of the score as a function of the number of the trial for individual animals, or average values of the score for groups of animals. Since different animals may reach a given norm of mastery after different numbers of trials, a modified procedure has been proposed by Vincent[610] so that the curves of different animals or groups of animals may be more readily compared. Loucks[360] has criticized the Vincent method and has suggested a somewhat different procedure. A description of both of these procedures has been clearly described and illustrated by Munn[433] and need not be repeated here.

- I. Characteristics of maze behavior:
  - A. *In initial stage:*[137, 136, 132, 161, 48]
  - B. *In fixation stage:*[127, 290, 649, 48, 656, 302, 524]
- II. Maze-learning as a function of the following factors:
  - A. *Biological factors:*
    - 1. Bio-chemical factors (including drugs)
      - a. Basal metabolism[253]
      - b. Drugs:
        - Alcohol[608, 408]
        - Morphine derivatives[528, 154]
        - Ureides[445, 650]
        - Miscellaneous drugs[363, 362, 512, 574, 473, 361]
      - c. Endocrine secretions
        - Adrenals[640, 641, 5, 364]
        - Gonads[354, 563]
        - Pituitary[364]
      - d. Inanition[500, 45]
      - e. Mineral factors[39]
      - f. Protein deficiency[38]
      - g. Vitamins
        - Vitamin A[389, 40, 410]
        - Vitamin B factors[410, 387, 388, 37, 459, 426, 400, 41, 550, 461, 544]
      - h. Miscellaneous conditions[575, 386, 390]
    - 2. Developmental factors:
      - a. Age[365]
      - b. Hereditary factors[472, 248]
    - 3. Neurological factors:
      - a. Chemical brain-constituents[6]
      - b. Cord mechanisms[298]
      - c. Cortical mechanisms[259, 345, 343, 254, 600, 601, 641, 602, 469, 266, 557]
      - d. Sub-cortical mechanisms[64, 187]
    - 4. Physical (external) conditions[204, 450, 217]
    - 5. Sex[420, 586]

B. *Psychological Factors:*
  1. Activity level[444, 510, 4, 85, 199, 86]
  2. Anticipation[549, 83, 79, 507, 192, 194]
  3. Habits
    a. Centrifugal swing[26]
    b. Direction-orientation[57, 31, 79]
    c. Forward-going tendency[31]
    d. Turning habits[521, 501, 504, 506]
    e. Wall-seeking-tendency[635]
  4. Motivation[145, 71, 72, 256, 437, 438, 349, 151, 501, 349, 73, 84, 470, 46, 75, 88, 385, 69]
  5. Orientation[669, 27, 588, 501, 317, 194]
  6. Pattern or structural features of maze[125, 24, 548, 505, 547, 661, 546, 312, 80]
  7. Previous runs[87, 139, 191, 589, 97, 230]
  8. Sensory cues[448, 101, 258, 451, 130, 179, 261, 135, 261, 53, 48, 92, 138, 274, 111]
  9. Series-characteristics[190, 655]
  10. Theoretically derived factors:
    a. Conditioning phenomena[287, 471, 678]
    b. Factors derived from factor-analysis procedures[594, 595, 587, 96, 609]
    c. Goal gradient[271, 501, 227, 82, 131, 441, 81] (cf. II. B. 2. Anticipation)
    d. Habit-family hierarchies[272]
    e. Least effort[636]
    f. Theoretical learning curves[649] (cf. [203])
  11. Transfer[303, 25, 503, 589, 611, 97, 607]

(*d.*) *The Problem-Box Method*

A fourth method for investigation of learning which is frequently differentiated is that called the Problem-box method. Investigators who have employed this method have been interested for the most part in the initial stage of learning referred to earlier, the stage of achievement or initial solution of the problem, rather than in the stage of fixation and stabilization. Our survey of problem-box situations may therefore be deferred until we reach the section on problem-solving behavior.

(*e.*) *Common Factors in Learning*

A number of investigators have tried, through the application of statistical technics, to determine whether there are common factors underlying different kinds of learning-performance. Since these studies involve the use of different kinds of learning situation, they cannot be appropriately classified in relation to any one method. Studies of this sort, with the rat as subject, have been reported by: Tryon,[593] Commins, McNemar, and Stone,[112] Tomilin and Stone,[587] Thorndike,[576] McCulloch,[307] Campbell,[96] Hunter,[287] Graves,[197] and Vaughn.[609]

MOTIVATION

Observation of the specific directions which the activity of the rat, or any other animal, may take under various conditions has led to the conclusion that much of its activity, like that of the human being, is regulated by specific needs which occasion activity directed to the satisfaction of such needs. It is thus possible to deal with the phenomena of motivation in the rat, as in the human being, and an important field of research upon the behavior of the rat has been concerned with such phenomena of motivation. Before going on to consider the technics which may be used to investigate such phenomena, it will be helpful to introduce a number of concepts which facilitate the discussion of phenomena in this field—the concepts of drive, goal, incentive. obstruction, and frustration. The *drive* may be defined as a condition in the animal which leads to activity directed toward a particular end-result. The activity may be spoken of as directed in the sense that persistent and varied activity of the animal takes place until that particular end-result occurs. The end-result, or terminal-act, which leads to the cessation of the animal's directed activity may be called the *goal.* The object which satisfies the need and thus makes possible the goal-activity is called the *incentive, e.g.* food. water, etc. (cf.[632]). An animal in a given situation may find its activity in the direction of a given goal interfered with, and so we may introduce the concept of an *obstruction* which impedes the attainment of the goal. Interference with aroused drives may be designated as *frustration.*

The relations between certain of these concepts may, perhaps, be clarified by regarding the adjustive behavior of a specifically motivated animal as somewhat analogous to the flow of a current in an electric circuit under the "drive" of an electro-motive force. If, in a circuit containing a battery, a resistance, and a key, the key is closed, a current will be found to flow around a circuit. The *drive* of motivated behavior may be regarded as analogous to the potential difference or electro-motive force of the electric circuit. It may be conceived of as a condition of disequilibrium in the organism which tends to produce activity of the organism in a given direction. Like the electromotive force, the drive may be said to possess both direction and magnitude. The *obstructions* which may impede such motivated activity may be considered analogous to the resistance or impedance which impedes the flow of electric current.

Technics for Measuring Strength of Drives

Four types of method for measuring the strength of various drives in the rat may be differentiated: the activity methods, the choice method, the obstacle method, and the learning method.

Activity Methods. *Frequency Method.* The frequency method obtains a measure of the strength of a given drive through a count of the number of times that some particular kind of behavior regarded as representing the operation of the drive occurs in a standard test-interval. Thus the strength of the male sex drive has been measured by counting the number of times a male rat will copulate with a female in heat during a standard interval (cf. Stone[560]). The frequency method mav be regarded as the quantitative form of the *presence or absence method.* The latter method is used to determine whether or not a given drive is operative in an animal on the basis of the presence or absence of behavior regarded as characteristically induced by that drive.

General Activity Methods. The methods already described for measuring general level of activity may be used to provide an index of the strength of a given drive if supplementary evidence is available to indicate the specific drive that is operative. The possibility of its use depends on the fact that the presence of a given drive in the animal may have as one of its consequences an increased level of general activity, even though no specific incentive related to that drive is available in the experimental situation. The measurement of general level of activity relative to a standard level may thus provide an index of the strength of motivating factors of some sort but cannot in itself indicate the particular drives involved. Supplementary lines of evidence must be obtained through other types of study to indicate the specific drive operative. Examples of such supplementary lines of evidence are data on basic needs of the animal that have not received satisfaction recently, the participation of specific bodily mechanisms known to be factors in particular drives, and supplementary determinations of the operation of specific drives by some one of the more direct methods.

Dorcus[144] has described an ingenious method which, like those just considered, obtains a measure of the strength of drives in terms of the amount of activity exhibited by the animal. It differs in that it provides, in addition, evidence of the incentive toward which the animal's activity is directed. The rat is placed in a vertically revolving activity-drum. Located on opposite sides of the drum and a little distance away are two cages containing different incentives. The turning of the activity drum causes the incentive-cages, by way of pulley and cord arrangements, to move toward the activity drum. When they get within a certain distance, the door in one of the incentive-cages is released by a trigger and the rat may enter this cage from the activity-cage. "If the incentive is not to its liking, it may re-enter the activity-cage and run until the other trigger is released." Sufficient details are not given to permit an adequate evaluation of the method. The report is of value, however, in suggesting a way in which the activity method may be made to indicate more specifically the nature of the drive determining the activity.

The Choice Method. The choice method obtains a measure of the relative strength of two different kinds of drive by presenting the animal with two different kinds of incentive and determining to which of them the animal moves. Thus, as in an investigation of Tsai, the rat may be deprived of food for some time to elicit a "hunger-drive," and of the opportunity for sexual-activity to elicit a "sex-drive." The rat is then placed in an apparatus (Fig. 123) which permits it to see food at the end of one path, and a rat of opposite sex at the end of the other. The route which the rat takes is taken as an index of whether the hunger drive or the sex drive is the stronger. Details of the technic and apparatus may be found in reports by Moss,[417] Tsai,[597] and Jenkins, Warden and Warner.[625] An analysis of the technic by Warden leads him to conclude that its many limitations make it unworthy of further development.[625]

Obstacle Methods.* *Resistance Method.* In the resistance method an effort is made to measure the strength of an animal's drive in the direction of a given incentive by determining the magnitude of an obstacle it will overcome to reach that incentive. In the apparatus devised by Moss[417] for this purpose, the obstacle is provided by an electrical grill which the animal must cross and the voltage of which may be progressively changed. By measurement, in electrical terms, of the maximum strength of the shock which the animal will endure in order to reach the incentive, an index is obtained of the strength of the drive. Warden has concluded that this technic, too, has numerous defects which make it unsuitable as a general technic for measurement of the strength of animal drives.[625]

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* For a discussion of terminology in relation to the obstacle methods, see Warden (625, pp. 8-10).

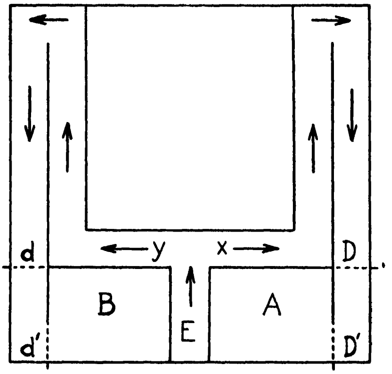

Fig. 123. Floor Plan of Tsai Choice Apparatus.

F, entrance, x, y, routes to incentive-chambers, A, B, incentive-chambers, d, D, doors to prevent retracing by test animal; d' D', doors to prevent exit by incentive-animal in A or B The walls of the incentive-chamber facing the alleys x and y are made of wire mesh, so that the animals tested may easily sense the stimuli in each chamber (From Tsai[597]) (By permission of The Williams and Wilkins Co , publishers of The Journal of Comparative Psychology)

Obstruction Method. The obstruction method shows certain similarities to the method just described, but differs in the fact that the obstacle used is not varied in magnitude in different trials, but held constant at a previously determined optimal value. The estimate of the strength of the rat's drive is based on the nature of its behavior in relation to the obstacle rather than on the magnitude of the obstacle which it will overcome. Thus one index of the strength of the drive is the number of times the rat will cross the grill to reach the incentive during a standard test-period, such as 20 minutes. The Columbia obstruction apparatus[625] designed for the use of this technic with rodents is reported as lacking many defects found in the earlier methods described above. The general plan of the apparatus is illustrated in Figure 124. Three compartments are arranged in series, a beginning compartment, A; an obstruction passageway (electric grill), B; and a final incentive compartment, C. The animal to be tested is placed in the entrance-compartment, A, and the incentive placed in compartment C. One or more preliminary trials, with the grill uncharged, is allowed the animal, so it may become acquainted with the nature of the incentive. Then one preliminary trial is given with the grill charged, following which the standard test period is begun. Tests of the reliability of this method have been reported by Warden,[625] by Stone and Tomilin,[566] and by Graves.[196] All studies agreed in showing a relatively high order of reliability. Technical details concerning tests of the validity of the method and the optimal conditions for its use may be found in Warden's monograph.[625]

Freeman[176] has recently given a preliminary description of an obstruction box designed to permit the use of different types of obstruction and to make unnecessary the reintroduction of the test-animal into the starting chamber, after each passage across the obstruction. The run-way for the rat, roughly circular in shape, is divided into eight sections connected in series. In each of these sections, an obstruction may be located, with the incentive just beyond. The rat may thus be tested successively on a series of obstructions without the need of being reintroduced each time to an entrance compartment. A number of alternative procedures for scoring the rat's behavior in the apparatus are proposed, but no data are given on the reliability or validity of these scores as indexes of the strength of the drives involved.

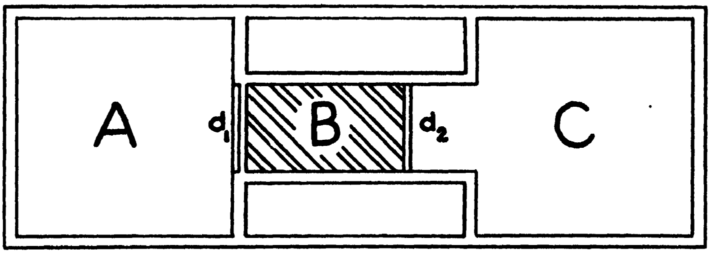

Fig. 124. Diagram of Floor Plan of Columbia Obstruction Box.

The test section consists of an entrance compartment (A) and an incentive compartment (C), each 10x10x12 in. in size, separated by a tunnel (B) with an electrically charged grill and a one-way door (d_2). The tunnel is 10 in. long and 4 in. wide. Its height can be adjusted for animals of different size. The grid extends from A three-quarters of the length of the tunnel. A manually operated sliding door (d_1) separates the entrance compartment from the tunnel. (Courtesy of C. J. Warden.)

A photograph of this apparatus and a statement of the procedure to be followed may be found in Warden, Jenkins, and Warner (628, p. 227). A floor plan of an earlier form of the apparatus is given in Warden (625, p. 18). (Reproduced from Warden. Animal Motivation, Experimental Studies on the Albino Rat, by permission of Columbia University Press.)

Learning Method. A fourth method which has been proposed for measurement of the strength of drives in the rat is the learning method.[628] Since the successful performance of a learning task may be regarded as a function of the drive involved, in addition to other factors, the rate or the efficiency of learning may theoretically provide an index of the strength of the drive, if other factors are held constant. The difficulty of adequately controlling other factors influencing learning has probably been responsible for the limited use of this method as an index of the strength of various drives. Investigations of the dependence of learning on factors of motivation may be found classified in the section on learning.

Special Drives

References to studies of the various special drives and the factors on which they depend are classified below:

I. Exploratory drive[51, 14]
II. Hunger drive
    A. Characteristics of eating or food-seeking behavior:[659, 55, 54]
    B. Food preferences (including abnormal appetites and cravings):
        1. Normal food preferences[225, 670, 671, 175, 672, 491]

2. Special appetites
  a. For carbohydrates, fats, and proteins[492, 484]
  b. For minerals[482, 487]
  c. For Vitamin B[490]
  d. For Vitamin D diets[648]
C. Food-seeking behavior as a function of the following factors:
  1. General food deprivation[55, 54, 58, 174, 250]
  2. Isolated nutritional components[228, 490, 492, 484]
  3. Organic factors[29, 599, 414, 482, 487]
III. Maternal drive
  A. Characteristics of maternal behavior:[313, 646, 403, 102]
  B. Maternal behavior as a function of the following factors:
    1. Biochemical factors[497, 495, 496, 347]
    2. Neural factors[34]
IV. Sexual drives
  A. Technics of measurement:[560, 561, 8, 516, 35, 21]
  B. Characteristics of sexual behavior:[564, 625, 36, 559]
  C. Sexual behavior as a function of various factors:
    1. In male rats
      a. Biochemical agents[113, 556, 558, 22, 11]
      b. Potency of male[562]
    2. In female rats
      a. Biochemical agents[240, 23, 645, 263, 11, 77, 153]
      b. Light-stimuli[633, 239]
      c. Reproductive organs[20, 238]
V. Thirst drive[74]
VI. Miscellaneous drives
  A. Wall-seeking tendency:[449]
VII. General or theoretical papers on motivation
  A. Theoretical papers:[156 155, 340]
  B. Strength of various drives:[626, 9]
  C. Relation of drives to other psychological phenomena:[536, 529, 626, 537, 10, 11, 580]

MOTOR PHENOMENA

Developmental Behavior Patterns

Fetal Behavior. The technics required in determination of the fetal behavior-patterns of the rat consist in, first, the operative procedures used in exposing the fetus so that its behavior may be observed and, second, special technics for facilitating observation of this behavior. Description of the technics involved may be found in reports by Angulo y González,[15, 16, 17, 18] and Swenson.[471] In recent studies, motion pictures have been used for recording the behavior of the fetal rats. Besides providing an objective record, this procedure has the advantage of making possible a leisurely and repeated observation of the behavior after the experiment has been concluded. Tilney has reported on the relation between the development of motor activities in the rat and stages in the development of the brain.[577]

Early Post-natal Behavior. Reports of the behavior of the rat in the first few days of life have been given by Small,[541] and by Anderson and Patrick.[7] Direct observation was the method used. The effects of early inanition upon certain performances in the developmental schedule have been reported by Biel.[46]

Energy-Expenditure

Crutchfield[122] has described an apparatus for measuring the energy-expenditure of rats in string-pulling in terms of the force exerted in pulling in a dish of food and the distance through which it is pulled. The energy-expenditure made on test trials was found to be a function of energy-expenditure required on previous habituation trials. Reports on related problems had previously been made by Adams[1, 2] and by McCulloch.[396]

Grasping

An apparatus for investigation of "grasping" in the rat has been described by Lei[350, 351] based on measurement of the length of time a rat can remain suspended from a horizontal wire. The animal is lifted up to the wire, in a uniform way, until both front paws come in contact with the wire. When the animal holds firmly to the wire, it is released quickly by the experimenter, but with care taken not to disturb the grasping response. An automatic device records the length of time the rat remains suspended. Chang subsequently investigated the dependence of the grasping reflex on certain neurological factors.[107]

Handedness

A method for determining handedness in the rat on the basis of the hand used to reach for food in a dish placed outside the cage was first described by Tsai and Maurer[508] and subsequently by Peterson.[453] A slight modification of this procedure was described by Milisen.[406] Peterson[455] also describes a different procedure for testing handedness in the rat, based on the hand used to operate a latch-box containing food. Investigations of the distribution of handedness-phenomena in rats and factors on which handedness depends have been reported by Peterson and his co-workers,[454, 456, 457, 458] Milisen,[406] Jasper,[306, 305] Kirk,[314] and Wentworth.[643]

Locomotor Activities

There have been a few studies reported in recent years on different aspects of the rat's locomotor activities: studies by Drew,[146] Morgan and Fields,[415] and Gilhousen[193] on the speed of locomotion, by Maier[374] on co-ordination of walking activity, and by Wever[644] and Morey[411] on swimming activities.

Work Capacity

Several investigations of the work capacity of rats under different conditions have been reported by Ingle and his co-workers.[249, 301, 206, 300]

PROBLEM-SOLVING BEHAVIOR

In the present section are considered those technics which are concerned with the behavior of the rat in solving new problems, i.e., in adjusting to situations for which its equipment of habitual responses is not immediately adequate. The term problem-solving implies that the animal placed in a new situation is impelled, under the influence of an internal drive, to act so as to reach an incentive of some sort, but is prevented from doing so directly by obstacles present in the situation. Consequently, the animal may be said to have a problem which requires solution. The concept of problem-solving behavior in work with animals may, in certain respects, be considered as analogous to the concept of thinking in the field of human psychology. Our survey of technics and investigations in this field is made in relation to the following three topics: (1) problem-situations; (2) characteristics of problem-solving behavior; and (3) problem-solving capacities.

Problem Situations

A classification, in alphabetical order, of various types of situations which have been used in setting new tasks or problems for the rat is given below. This list is not limited to the puzzle-box situations which are frequently described as illustrative of the problem-box method for investigating learning, but includes all types of situation which can be regarded as setting new problems for the animal. In terms of the characterization of a "problem" given above, maze-situations, discrimination-response situations, and even conditioned response situations may be regarded as providing new problems of adjustment for the animal. They have, therefore, been included in the list. For similar reasons are included situations designed to test the occurrence in problem-solving behavior of special phenomena, such as insight, reasoning, and the like. An effort has been made in the classification to characterize the different situations in terms of the performance that the rat is called upon to make in order to solve the problem.

Alternation Problem. The alternation problem, which was devised by Carr,[98, 99] required the rat, in a series of trials, alternately to select passageways on opposite sides of a box with all cues eliminated other than position of the passageway. The animal must go to the left on one trial, to the right on the next, to the left on the next, and so on. Variations in the problem occur by way of changes in the interval of time between successive trials.

A similar type of problem is provided by the temporal maze later described by Hunter,[285, 281, 294] and to which reference has already been made.

Delayed Response Problems. Under this heading may be classified situations in which the animal is prevented from responding to a given stimulus condition until an interval of time has elapsed. The solution of the problem depends on the capacity to make a particular response even after an intervening interval. This type of situation has been utilized principally in relation to the question of whether or not the animal has memory-ideas. It has been argued that, under certain conditions, the ability to respond correctly to a given stimulus-object after an interval of time is evidence of the utilization by the animal of a memory-idea of the stimulus-object. Descriptions of different types of apparatus for the investigation of delayed reaction in the rat may be found in the following references:[280, 284, 367, 257, 392, 629, 658, 652, 393, 394]

Discrimination-response Problems. For a survey of technics involved in the discrimination-response type of situation, see pages 202-210, 218.

Imitation Problems. A description of situations said to have permitted observation of imitative behavior in the rat has been given by Berry.[43] The question at issue was whether rats could be aided in the solution of a given problem through imitation of the successful behavior of other animals.

Insight Problems. Situations designed to test whether or not rats show "insight" in the solving of a problem have been described by Helson,[237] Hsiao,[265] Tolman and Honzik,[582] Gilhousen,[189] McDougall and McDougall,[400] Buytendijk and Fischel,[91, 92] and Hamilton and Ballachey.[218]

Maze Problems. A survey of maze technics is given on pages 218-227.

Multiple-choice Problems. In this type of problem, the rat is required to select one direction of response out of a multiplicity of more than two available, on the basis of previous trials which provide cues to correct response. This problem has been used quite extensively in relation to the question of whether or not animals possess ideas. Hamilton[216] has described a multiple choice apparatus for rats providing for four possible choices, and Burtt[89] one permitting nine possible choices. The problems set the animals in Hamilton's apparatus are of special interest in that the problems are not solvable. Nevertheless the behavior of the animal can provide cues to certain factors regulating its problem-solving behavior. The method shows similarities in this respect to Krechevsky's use of the Stone multiple-discrimination apparatus for investigation of "hypothesis formation" in rats. Atkins and Dashiell[19] have described a temporal multiple-choice problem for rats, which requires the animal to move to one of four available doors depending on the temporal order in which they are illuminated.

Obstruction-box or Puzzle-box Problems.* Classified in this section are problems in which the task set is that of getting into or out of a box, the rat being prevented from doing so by an obstruction of some sort. Food (placed inside or outside of the box) usually provides the incentive. The problem for the animal is to find a way of removing the obstruction. Two of the earlier forms of apparatus used in this field are considered first. Different types of apparatus differ in the nature of the obstruction and the means available for removing it. A useful analysis and classification of different types of apparatus in this field has been given by Jenkins.[307] A study of interest in connection with the problems classified here is that of Warden[621] who found that a preliminary period of feeding in the problem box served to cut down appreciably the length of time required by the rat to solve the problem.

Sawdust-obstruction Box. Watson[438] and Kline[316] have described simple obstruction-boxes in which sawdust is piled around a box, without a bottom, containing food. The problem is solved by the animal digging through the sawdust to come up into the box from the bottom.

Paper-obstruction Box. Kline[582] has described a simple apparatus in which the door is prevented from opening by a strip of paper sealed across it at one point. The animal must tear or bite off the paper in order to reach the food inside.

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* The term puzzle-box should possibly be used in preference to the term obstruction-box in order to avoid confusion with the obstruction method of measuring the strength of animal drives (cf. p. 230). The term obstruction-box, on the other hand, seems to indicate more clearly the nature of the performance required of the animal in the situation, that of removing the obstruction to the goal.

Latch-obstruction Box. In an early form of the latch box, described by Watson,[638] a latch, which must be pushed up by the animal, prevents the door from opening. Food is located inside the box. McDougall and McDougall[390] describe a multiple-latch box in which the rat is required to operate in succession as many as 14 latches in a specific serial order. Lashley[336] described a series of latch-boxes of different orders of complexity designed for the investigation of problem-solving behavior.

Platform (or Plate) Problem-boxes. In this type of apparatus, the door separating the rat from the food remains closed until the animal steps on one or more levers or platforms at a greater or less distance from the door. This type of problem is apparently more difficult for the rat than those in which the object to be actuated or moved stands in close spatial relation to the door. The use of platforms or plates to open the door has the advantage that the motor performance required is not dependent on manipulatory capacities but only on the capacity for locomotion. It therefore is useful for comparative investigations of different animal-forms.

(a) *Single-platform Boxes.* In single-platform boxes, the animal must stand on or operate but a single platform in order to bring about the opening of the door to the food-box. Devices of this sort have been described by Watson,[638] Bassett,[30] and by Stone.[554] Stone reports his apparatus has a reliability on odd versus even trials of 0.35 to 0.88. This coefficient is regarded as sufficiently high to justify comparison of groups of animals but not of individuals.

(b) *Double-platform Problem Boxes.* In double-platform boxes, two platforms located at different positions relative to the door are involved. Such boxes have been described by Lashley[333] and Gengerelli.[180] Lashley's double-platform box is illustrated in Figure 125. The rat must step on the platforms in a particular order to solve the problem. In Gengerelli's problem box, the order does not matter.

(c) *Multiple-plate Apparatus.* A device involving a series of 3 plates located in the floor in the general neighborhood of the door has been described by Jenkins.[307] The plates serve as levers in the same way as the platforms in the devices described above. This apparatus has the merit that problems of different degrees of difficulty can be set for the animal. Thus the experimenter may control the number of plates that the animal must actuate, and the serial order in which they are to be actuated. The ground plan of the apparatus is shown in Figure 126. Food placed inside the food-box, blocked by the closed door D, is used as an incentive. In the modification of the apparatus described by Riess,[498] copper strips are attached to the plates so that the animal may be given an electric shock when it incorrectly depresses any platform. An external control box permits the severity of the shock to be controlled by the experimenter. In his investigation of the comparative problem-solving ability of rats and guinea pigs with this apparatus, Riess[498] found that the limit of the rat's capacity was represented by the two-plate problem. In this problem, plates 1 and 2 had to be depressed in sequence in order for the door to the food-box to be opened. Nine perfect trials in a series of ten were used as a norm of mastery. Only 33 per cent of the group of 35 rats examined succeeded in this problem, but none of the guinea pigs. In the one-plate problem, 69 per cent of the rats succeeded. Constructional details for the Jenkins problem-box, as designed for rats, may be found in the reports of Jenkins and of Riess.

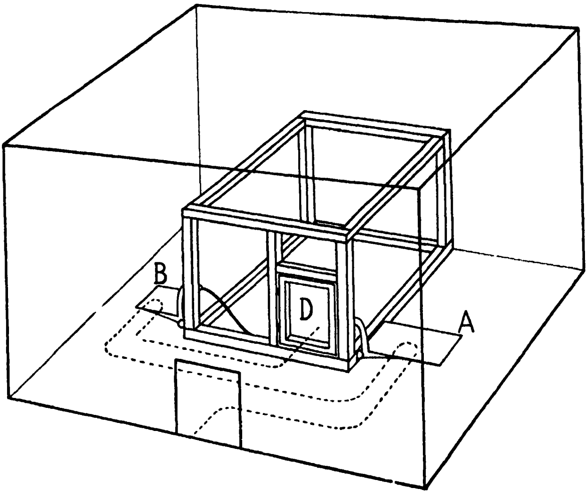

Fig. 125. Lashley Double-Platform Problem Box.

The door D is opened when the platforms A and B are successively pressed down. [From Lashley,[333] Brain Mechanisms and Intelligence By permission of the publishers, The University of Chicago Press.]

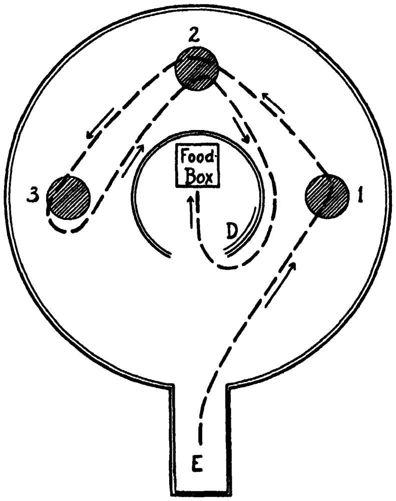

Fig. 126. Floor Plan of Jenkins Triple-Plate Problem Box.

E, entrance compartment (door from it to main cage is not shown in diagram) The door D is opened when the series of plates have been depressed in a particular order. Broken line indicates route to be taken in the 4-step problem, in which plates must be depressed in the order 1, 2, 3, 2. [Figure from Warden, Jenkins, and Warner,[628] Comparative Psychology, Principles and Methods The Ronald Press Company, Publishers]

Reasoning Problems. Maier describes a series of situations to determine if the rat is capable of reasoning.[360, 370, 376] The references cited are those in which various tests of reasoning ability were initially described. Other papers on reasoning ability, which will be cited below, utilize some one or more of these same reasoning problems. Maier characterizes the ability to reason as a capacity to bring together, or combine, spontaneously, elements of past experience which have not previously been associated by contiguity.[370]

An illustrative problem (the three-table problem) designed to test for the presence of reasoning capacity in the rat is the following: A central junction point is connected by separate elevated pathways to each of three tables X, Y, and Z. Each table is of a different shape, and is screened at one end so that its shape and any objects on it may not be seen from other parts of the network. The rat is first permitted to explore the entire situation (Experience 1). It is then placed with food on one of the tables (e.g., table X) and permitted to eat for a brief interval. It is disturbed occasionally in order to lead it to become acquainted with the characteristic features of the table (Experience 2). Now, in the *test trial*, the rat is placed on one of the other tables, e.g., table Y. The question is, will the rat go immediately to table X or will it reach there only after a preliminary journey to the other table? If, in a large series of such trials, sufficiently varied so factors of learning do not enter, the rat's percentage of immediate solutions is well above chance, the conclusion is drawn that the rat has demonstrated the ability to reason.[370] Another reasoning problem used in a number of Maier's studies is one in which the rat is required to combine the separate features of four separate experiences.[376] Wolfe and Spragg[660] have criticized the technics used by Maier to demonstrate the occurrence of reasoning in rats, and Maier[373] has in turn called attention to certain features of his technic which he states were not take into account by his critics.

In addition to the situations which have been explicitly labelled as tests of reasoning, others may be considered as requiring similar types of performance on the part of the animal, e.g., those designed to test for the presence of "insight."

Characteristics of Problem-Solving Behavior

General Course of Behavior. In this section are listed reports which describe the general course of behavior of the rat in situations requiring new adjustments, and characterizations of adjustive behavior not readily classified in the following section on special adjustive phenomena. (References to reports on problem-solving behavior in discrimination-response and maze-situations are given in the sections dealing with those special methods.) Reports on adjustment in free field situations are included here. The field situation is one in which there is a minimum of restraint imposed on the rat's behavior by the structure of the apparatus. Typically, a motivated animal is placed in an open enclosure containing a reward in some portion, and the course of his adjustment observed.[213, 234, 235, 76, 158, 613, 604, 605, 667, 44, 359, 121]

Special Phenomena. Classified in this section on special phenomena are studies designed to determine whether or not some specific phenomenon plays a rôle in the problem-solving behavior of the rat. A list of such special phenomena and references to papers in which they are reported follows:

1. *Concept-formation*[10, 11, 168, 169, 170, 171]
2. *Insight or "Umweg" behavior*[218, 311, 226, 329]
3. *Hypothesis-formation*[319, 320, 321, 657]
4. *Memory phenomena*[393, 394, 629, 658] (See also Section 7 below on Symbolism and abstraction.)
5. *Plasticity and variability*[221, 220, 219, 323, 324, 325, 52, 656]
6. *Reasoning*
   a. Tests of occurrence of reasoning in rats[523, 660, 373, 376]
   b. Theories of reasoning[273, 375]
   c. Reasoning as a function of the following factors
      Characteristics of the problem situation[369, 381, 384]
      Maze-learning ability[358]
      Neural factors[370, 371, 372, 382, 377, 378]
      Nutritional factors[642]
7. *Symbolism and abstraction*[651, 652, 653, 50]

Problem-solving Capacities (Intelligence Level)

In the present section are classified investigations concerned with determinations of the problem-solving capacities of individual rats, or groups of rats. The emphasis in such studies is on properties of the organism that determine problem-solving performance rather than on the description of the behavior involved in the performance. The specification of the limits of an animal's ability to solve different kinds of problems is sometimes referred to as a measurement of its intelligence-level. Since, in the determination of this level, the animal's capacity to solve a wide variety of problems may be tested, it is apparent that the intelligence level cannot, without experimental demonstration, be regarded as dependent on any one specific mechanism. The term must be regarded as a class-name embracing a variety of different capacities. The extent to which these may depend on common factors is a problem for experimental research.

A. Determination of problem-solving capacities:[498, 252, 393, 629]
B. Problem-solving capacities as a function of the following factors:
   1. Biochemical factors[39, 38, 37]
   2. Developmental factors:
      a. Age[368]
      b. Hereditary factors[433, 596, 248]
   3. Neural factors[370, 220, 274, 218, 596, 433, 378, 469]
   4. Sexual factors[402, 586]

PSYCHOPATHIC PHENOMENA

In recent years, there has been an increasingly strong interest exhibited in the development of technics for producing in animals behavior-phenomena resembling those which occur in psychopathic states in man. It has been felt that the development of such technics might make it possible to investigate factors which are not readily investigated in the human subject (cf. Liddell[355]). The rat, as well as other kinds of animal, has served as subject. The terminology used for designating maladjusted behavior in animals is as yet not very definitely standardized. Experimental neurosis, conflict-behavior, frustration-phenomena are representative of the terms most frequently used. A number of general papers of interest in relation to the question of behavior-disorders in the rat have been published.[207, 476, 355, 115, 420]

Technics for Producing Experimental Neurosis

The term experimental neurosis has not received any final and generally accepted definition. The significance of the term and suggested criteria have been discussed by several writers.[114, 380, 420] In the present discussion, the term may be taken to mean behavior-activities or traits, produced experimentally in animals, which resemble those occurring in abnormal or psychopathic states in man. The term is thus not limited to behavior-symptoms analogous to the *neuroses* in man, as distinguished specifically from the *psychoses*, but may be regarded as potentially including symptoms in the entire field of psychopathology. Since there seem to be no precise standards for differentiating abnormal from normal behavior in human beings, it is not surprising that similar difficulties have arisen in attempts to make a like distinction in the field of animal behavior.

Work upon experimental neurosis in the rat has so far been directed chiefly to finding ways in which it might be produced. Cook, Maier, Humphrey and Marcuse, Morgan and Morgan, and Hall and Martin have described situations by means of which they were able to produce behavior changes designated as "experimental neuroses." Other reports have been made of behavior-changes regarded as more or less abnormal, but which did not meet the criteria established by the author for "experimental neurosis."

Conflict Situations. *Conditioned-response Technic.* Cook[114] defines experimental neurosis as "any chronic abnormal behavior, experimentally produced." He reports success in developing this type of behavior in 3 out of a group of 6 albino rats subjected to a number of different kinds of experimental situation. Four types of situation were tried; only one was effective in producing changes in behavior regarded as meeting his criteria of experimental neurosis.

The final situation and procedure adopted was as follows: "The rats were strapped to a stand so that the only sizable limb-movement possible was flexion of the right fore-leg. Under certain conditions such a flexion was rewarded with a food pellet, under other conditions, it was punished with an electric shock."[114] By means of a procedure modelled on the conventional conditioning technic the rat was trained to discriminate between a strong and a weak light placed in front of it—to respond with flexion of the fore-foot to a bright light, and to refrain from this response when a faint light was presented instead. After this discrimination was well established, the critical series was begun. The intensity difference between the bright and dim lights was gradually decreased until the limit of discrimination was reached. This procedure led to behavior which met Cook's criteria of experimental neurosis in 3 out of 6 subjects. The length of time required for this procedure is not definitely indicated.

The symptoms regarded as neurotic included both changes in the behavior of the animal in the conditioning situation, and changes exhibited by the animal in its living quarters. The three neurotic animals did not show identical symptoms. Rat No. 1, for example, showed the following disturbances: "(1) An increasing tendency to react to the problem by a regular but temporary loss of the capacity to inhibit flexions of the foreleg. (2) Spontaneous, loud squealing accompanied by general body and tail-stiffening. (3) An exaggerated jumpiness and tendency to stiffen when touched. (4) A practice of inhibiting postural reflexes (holding poses) for long periods when in contact with the experimenter's hand. (5) A tendency to squeal when touched or lifted. (6) An active avoidance of the experimenter's hand in the living cage." Rat No. 2 showed the following changes: "(1) An increasing tendency to react to the problem with a regular, but temporary, loss of the capacity to flex the foreleg. (2) Greatly decreased activity in the cage, including sleepiness and an indifference to certain stimuli normally adequate to excite exploratory behavior." Rat No. 3 showed: "(1) The loss of a previous habit of eating while strapped in the apparatus. (2) Struggling in the apparatus so continuously and so violently that further experimentation was useless," and in addition, the second symptom specified above for rat No. 2. Cook concludes, on the basis of his analysis of the situations that failed to produce neurosis, that an essential feature of the neurosis-producing situation is "that activity other than that of the responses utilized in the stressful situation be very limited."

Discrimination-apparatus Technic. Maier[380] made use of the Lashley discrimination-technic (jumping technic) in trying to produce neurotic behavior. He concluded from an analysis of reports of experimental neurosis in large animals (the dog, sheep, and pig) that the essential factor in the effectiveness of the conditioning procedure lay in the arousing, by a given stimulus, of two incompatible tendencies to response, without a third possibility being available. The apparent inadequacy of the discrimination technic he attributes to presence of a third possibility for response. He therefore proposed to alter one of the standard discrimination procedures so that a third possibility for response would be excluded.

The following procedure, the one-window situation, is representative of the type of situation found to be effective. The animal was first trained in a Lashley jumping apparatus, in the usual way, to discriminate between two stimulus-patterns. If the animal jumped against the card bearing the form regarded as correct, the card would fall back and the rat would obtain food on the platform just behind. If the rat jumped against the negative card, collision with the rigidly fixed card would lead to a fall into the net. After the discrimination had been established, a one-window series was introduced. When the negative pattern was presented at one window, the other being blocked by a neutral card blending with the background, the rat was impelled by its previous training to refrain from jumping. It was, however, stimulated to jump by a strong jet of air turned upon it from behind, and was prevented from escaping from the apparatus by obstacles placed at appropriate places around the jumping platform. Certain of the experimental animals placed in this type of situation developed behavior-changes designated by Maier as experimental neurosis. Nine out of a total of 22 animals were thus affected.

The time required to establish such neurotic behavior varied markedly in different animals and with the specific situation used. In all but one animal, it was found possible to induce a considerable series of attacks beyond the initial one.

The symptoms of experimental neurosis exhibited by Maier's animals differ markedly from those reported by Cook. Maier refers to the set of symptoms produced as "the neurotic pattern" and characterized it as a specific and violent pattern of behavior. The pattern shows two phases, an active and a passive phase.

The active phase begins characteristically with a sudden leap by the animal from the jumping platform to the floor. On reaching the floor the animal runs about at a high rate of speed, usually in a circular path, often colliding violently with walls and table legs. This stage may be followed by a convulsive stage which may take somewhat different forms. The animal may lie on its side, with legs kicking and tail spinning; or take a sitting posture with legs clawing the floor; or, in the sitting posture, exhibit a violent trembling of the entire body. A fourth stage of vigorous hopping may follow, and the active phase terminate with tic-like movements of the forelegs or head.

The passive phase follows. The violent movements subside and the animal becomes unresponsive. It may be picked up and placed in various postures which will be resisted by the normal rat and "will hold these postures sometimes for minutes at a time." A gradual recovery period in which the animal increasingly resists manipulation finally terminates the passive phase. A further characteristic symptom of the neurotic animal which appears later is chronic retiring behavior in the living cage.

Maier distinguishes from this neurotic pattern less extreme behavior modifications which may precede the neurotic "attack" in the neurotic animals, and may also occur in the rats that fail to exhibit the specific neurotic pattern. These behavior changes are referred to as "nervousness" and consist in symptoms such as chattering of the teeth, refusal to eat, and avoidance of the situation. This "nervousness" was regarded as exaggerated normal behavior and not a qualitative difference in behavior such as represented by the neurotic pattern.

Direct Stimulation Technics. Morgan[416] and Humphrey and Marcuse[276] report the production in rats of neurotic patterns of the Maier type through the use of procedures differing markedly from those used by Maier. We refer to these as "direct stimulation" technics since the effects seem to depend on the direct use of disturbing auditory stimuli rather than on the preliminary use of training procedures.* It is quite possible, however, that in terms of the psychological situation faced by the animal, the situations are similar. Humphrey and Marcuse describe two types of situation: the moving food-box situation, and the bell-series of disturbing stimuli.

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* At Morgan's suggestion, the term *audio-genic seizure* is now generally applied to the behavior-deviations resulting from such auditory stimuli.

It is of interest to note that Donaldson reported many years ago on the effectiveness of certain auditory stimuli in producing behavior that would probably be designated today as experimental neurosis. In commenting on the acuteness of sensation in the rat, he states "the response to some sensory stimuli, especially to sounds, is very marked—as is shown by jumping when the hands are clapped, and in some cases by an almost maniacal running and jumping—going on to exhaustion—when a bundle of keys is jingled before a cage."[141]

Air-Blast Situation. In one part of his study, Maier examined the rôle of the air blast in producing the neurotic pattern by subjecting a small group of animals to the noise associated with escaping air. He concluded from these experiments and others in which the sound of jingling keys was used, that "the view that the neurotic pattern is released by direct stimulation may account for a few of our cases, but it in no way accounts for the attack of the majority of them."[380]

Morgan and Morgan[416] report somewhat later, on the basis of experiments performed before Maier's detailed report was available, that they were able to produce abnormal behavior of the Maier type by exposing rats to the auditory stimulus associated with an air blast. The air was not directed toward the animals and the source was located several feet away. The abnormal behavior, which occurred in 10 per cent of the animals tested (4 out of 41), was therefore attributed to the auditory stimulus involved. The authors suggest that the neurotic behavior reported by Maier may also be a consequence of the air-blast stimulus and not of the other features of Maier's procedure.

Hall and Martin[214] also report on the effectiveness of the air blast in producing abnormal behavior of the kind described by Maier, and make suggestions for standardizing the experimental conditions. The rat is placed in a circular enclosure 7 feet in diameter, and compressed air released through a tire air-chuck for a period of two minutes. Attacks were produced in 43% of the sample of 51 animals used.

Moving Food-box Situation. The initial situation used by Humphrey and Marcuse may be called the "moving food-box situation." Rats were trained to run a Warner-Warden multiple-Y maze for a period of 25 days. The food-box used had no bottom, so that its contents were in direct contact with the floor of the room. After the rat had entered the food-box, the box, together with the enclosed rat, was moved about over the floor for a distance of 4 to 10 feet.

The effect of this procedure on the animals with whom tried was to induce what the authors call "chronically disordered behavior." Among the symptoms of this disorder were: inferiority to the control rats in maze-learning scores, "belly-crawling" in the maze, loud teeth-gnashing, spasmodic starts from side to side in cul-de-sacs, shivering, biting of metal mesh and door of food box, and hesitant behavior on reaching food box. None of these symptoms was observed in four control animals which were not moved about after completing the run in the maze.

Bell-series of Disturbing Stimuli. Another type of situation was furnished by the following series of stimuli, regarded as of progressively increasing degrees of severity. Stimulus No. 1 "consisted of ringing an electric bell . . . for 3 minutes on floor of the carton on the upper surface of which the animal was placed." Stimulus No. 2 "consisted of ringing the bell for 3 minutes close up to the cage, and on the same plane with it;" Stimulus No. 3 consisted "in swinging the cage gently 5 or 6 times with an oscillation of 180° while the bell was ringing in the position of Stimulus No. 2." Through the use of stimuli in this series, the authors succeeded in producing seizures of the Maier type in a total of 15 out of 79 rats or in 19 per cent, in the initial trial of the various stimulus-grades tried. Evidence of the greater susceptibility to neurosis of the "chronically disordered rats" is shown by the fact that Stimulus No. 1 was effective in producing seizures in 3 out of 6 rats, or 50 per cent. This stimulus was effective in normal rats in only one out of a total of 50 rats tested. Stimulus No. 3 was much more effective, since it produced seizures in 11 out of 23 normal rats tested, or almost 50 per cent. The symptoms observed were essentially the same as those described by Maier, including both the active and passive phases. The active phase begins with violent jumping around the cage, and then, after the cage-door is open, follows the course already described.[276]

Technics Failing to Produce Neurosis

Following is a brief summary of procedures which were regarded by their inventors as having failed to elicit "experimental neurosis" although some were said to produce certain abnormalities of behavior.

Before designing the conditioned-response situation described above, Cook tried a series of three somewhat similar situations in which the freedom of movement of the animal, in other than the critical response, was less rigidly restricted. Here, too, the animal was first trained to make two different and incompatible types of response to lights at the two extremes of the brightness scale. The difference in brightness between the positive and negative stimuli was then gradually decreased. The failure of neurotic behavior to develop was attributed by Cook to the availability of other modes of response than those to which the animal was trained.

In some of his experiments, Maier used an apparatus and procedure exactly like that described earlier, with the one difference that an electric grill, located on the jumping platform, was used to induce the rat to jump when "resistance" to jumping developed, instead of a stream of compressed air. This procedure, by itself, proved ineffective in establishing the neurotic pattern. Why the air was a more effective factor than shock in precipitating the neurotic attacks is as yet not clear from the experiments so far performed.

The use of a moving food-box by Humphrey and Marcuse to induce "chronically disordered behavior" has already been described. It proved ineffective by itself in inducing attacks of the sort designated as experimental neurosis.

Phenomena Resembling Psychopathic Symptoms

A number of reports have been made of forms of behavior in the rat, which, while not considered instances of experimental neurosis, resemble symptoms of behavior found prominently displayed in the human disorders. A classification of such references is given below.

Activity level[277]
Aggressive behavior[126, 443]
Competitive behavior[352]
Emotional behavior (See section on this topic)
Frustration phenomena[408, 407, 409, 279, 555]
Stereotyping of behavior[211]
Regression[514, 442, 47]

SOCIAL PHENOMENA

There have been relatively few attempts to investigate social phenomena in the rat. Those that have been made have been reported in quite recent years. Reference to the topic in Munn's monograph is limited to a few studies of imitative behavior.[433]

The Strength of the Social Drive

Locke[357] and Bayroff[31] devised technics for measuring the strength of a social drive in the rat as indicated by the tendency of an experimental animal to go to a compartment containing one or more other animals. Locke used the Columbia obstruction method for obtaining a measure of this tendency and Bayroff a choice method (cf. pp. 229, 230). In Locke's arrangement, the experimental animal's home cage, containing a familiar cage-mate, was connected to the usual incentive compartment and served as the incentive. Appropriate procedures were adopted for excluding the interference of other drives, and the experimental animals were tested after varying intervals of separation from their cage-mates. No evidence of a special social drive was obtained under these conditions. Locke proposes a number of possible variations in the conditions that might be effective in producing more positive results.

In Bayroff's application of the choice method, alternative choices provided for the experimental animal were, in one part of the experiment, a compartment containing other rats and a compartment containing food, and in another part of the experiment, an empty compartment and one containing other rats. Bayroff points out certain defects in the technic, such as the difficulty of excluding the effects of position habits with the apparatus employed.

The Dependence of Psychological Phenomena on Social Factors

A number of additional procedures, beyond those mentioned above, have been described for determining the effect which the presence or activities of other animals may have on a given psychological phenomenon. Thus Harlow[224] investigated the effect of social factors on eating activity, Bruce[76] and Rasmussen[467] the effect of such factors on obstructed drinking activity, Lepley[352, 353] their effect on speed of locomotion, and Waters[630a] their effect on maze learning. The results of the various studies upon social phenomena in the rat have on the whole been rather indecisive. The studies reported provide a basis, however, on which more satisfactory technics might be developed.

TROPISTIC BEHAVIOR

According to Crozier, who, with his co-workers, has been responsible for much of the work on tropism in rats, the tropism may be defined as "oriented behavior in an energy field." The procedure used in the investigation of such oriented behavior has consisted in (1) placing the rat in a situation involving exposure to precisely measurable physical stimuli or conditions and (2) observation of the rat's behavior in relation to these stimuli. In the formulation of results, an interesting feature of Crozier's treatment has been the attempt at derivation of mathematical formulae for representing orientation of the rat as a function of the stimulus-conditions involved.

The types of situation so far investigated may be differentiated in terms of the types of stimulus-condition operative. These have included tactual, light, and gravitational stimuli. A description of the technics employed in the earlier studies in this field may be found in Munn's monograph.[433]

Since the time of Munn's survey, relatively few new studies have been reported. These are limited to further studies by Crozier and Pincus upon the analysis of geotropic orientation in the rat,[117, 118, 119] a study by the same authors on phototropic orientation in young rats,[120] and a study by Hamilton and Matlack[222] upon a procedure for modifying a "contact-tropism" of the rat. These authors wished to determine whether the tendency of the rat to come into physical contact with its cage-mates could be eliminated by training. The procedure used consisted in administering a shock to the experimental animal whenever it came in contact with a cage-mate, without at the same time shocking the cage-mate. This conditioning procedure proved to be effective.

References to orientation-behavior not regarded as tropistic in nature may be found in the section on problem-solving behavior and in that dealing with certain of the orientation-factors regulating maze-learning behavior (pp. 227, 239).

GENERAL LABORATORY TECHNICS

In the present section are classified references to reports of miscellaneous procedures and data which may be found useful in the rat-behavior laboratory. Descriptions or references to other relevant technics may be found in other parts of this volume.

Anesthesia[28, 527]
Electric shock[264, 148, 428, 423, 149, 174, 429, 241]
Marking rats[614, 551]
Observation technics[35, 671]
Surgical technics[109, 134, 67, 62, 117]
Treatment of pathological states[383],

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11

The Circulatory System

By

J. Q. GRIFFITH, Jr., and W. A. JEFFERS

Assisted by E. ROBERTS

THE HEART

Examination of the heart by palpation and auscultation is unsatisfactory in the rat because of the pronounced tachycardia. The normal pulse rate is about 300 a minute. Therefore, though the heart beat can be palpated by the fingers on the chest wall and the heart sounds can be heard through a stethoscope with the bell placed on the chest, individual heart sounds cannot be evaluated nor the pulse accurately counted. While electrocardiography has been practiced in rats for various purposes, its usual object is to count the pulse. Roentgenograms of the heart may be made for heart size, and these can be confirmed postmortem by measurements of heart weights.

In this chapter certain technics will be considered which are applicable to the normal as well as to the diseased animal. Then the spontaneous occurrence or the production of diseased states will be considered.

The Electrocardiogram. To obtain electrocardiograms we use the Hindle model, Cambridge Instrument Company. Needle electrodes are employed. The lead terminals are burnished with emery paper, and straight steel sewing needles 1½ inches long are secured to them by wrapping with pure silver wire. With the anesthetized animal lying supine the needles are thrust under the skin of the forearms and lower left leg. The usual three indirect leads used in human electrocardiography are thus obtained. (See Fig. 127.)

Cardiac Roentgenogram. Under anesthesia, the rat is fastened to a board by means of thumb tacks through each foot. After testing the x-ray setting, a small dental film is slipped under the chest just before making the exposure. A 6 KW x-ray tube is used. The target-film distance is 30 inches. A setting of 10 milliamperes and 80 peak KV for 6 seconds is satisfactory for a rat of 200 grams.

BLOOD PRESSURE

Indirect Method Using the Foot. This method, originally reported in 1934,[1] has undergone several modifications since that time. The original technic is as accurate as the one now in use, but somewhat more cumbersome. The test depends upon visualizing blood flow in the cutaneous minute vessels of the foot while pressure in a sphygmomanometer cuff about the thigh is raised and lowered. When the pressure in the cuff exceeds systolic pressure the flow will stop, to start again when the pressure is lowered. In an animal with a normal blood pressure these two points will be almost identical, i.e., where flow stops on raising pressure and starts on lowering pressure, though the second is always a little lower. However, in animals with some form of experimental hypertension flow may not start until the pressure is 30 to 40 mm. below the point at which it had stopped. This is attributed to inertia of the blood in the capillaries, so that it requires a little more pressure to start flow than to maintain it when once started. It is pos-

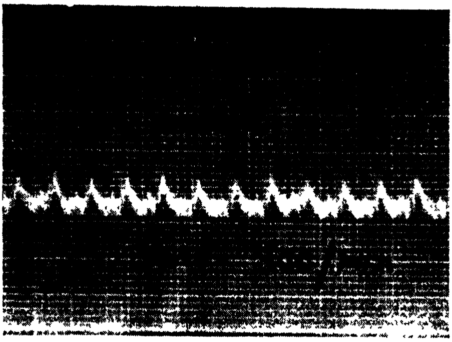

Fig. 127. Rat Electrocardiogram.

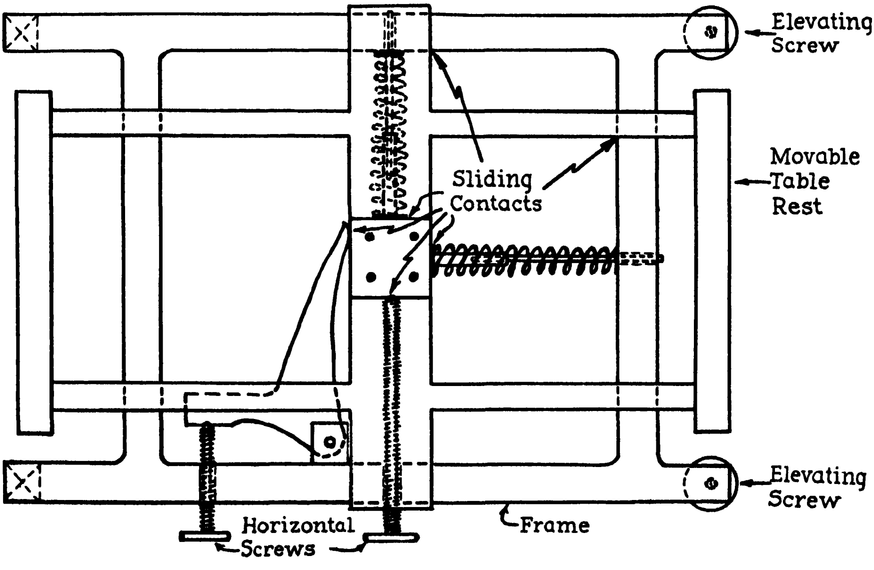

Fig. 128. Mechanical Stage.

sible that in hypertension the pulse wave may be sharp at the top, i.e., the time of maximal pressure may be of short duration. In such cases the inertia of the blood in the minute vessels introduces a distinct factor of error. Therefore, while originally we read the pressure at which flow started, we now accept the point at which flow stops as more nearly representing systolic pressure.

Lighting. Virtually any form of lighting may be used if sufficiently strong. Originally we used the light from a 500-watt lamp passing through a 500 cc. spherical flask filled with water. This serves to remove heat and concentrate light on the foot. This arrangement is quite satisfactory but requires frequent adjusting to keep the field well illuminated. For the past year we have used an Ultropak (Made by E. Leitz, Inc., 60 E. 10th St., New York City) which lights the field directly and is entirely satisfactory. Some observers prefer a blue or green light, which makes the capillaries appear dark and is more restful for the eyes. We sometimes use the green filter in the Ultropak or add a little methylene blue to the water in the flask if the original lighting system is being used.

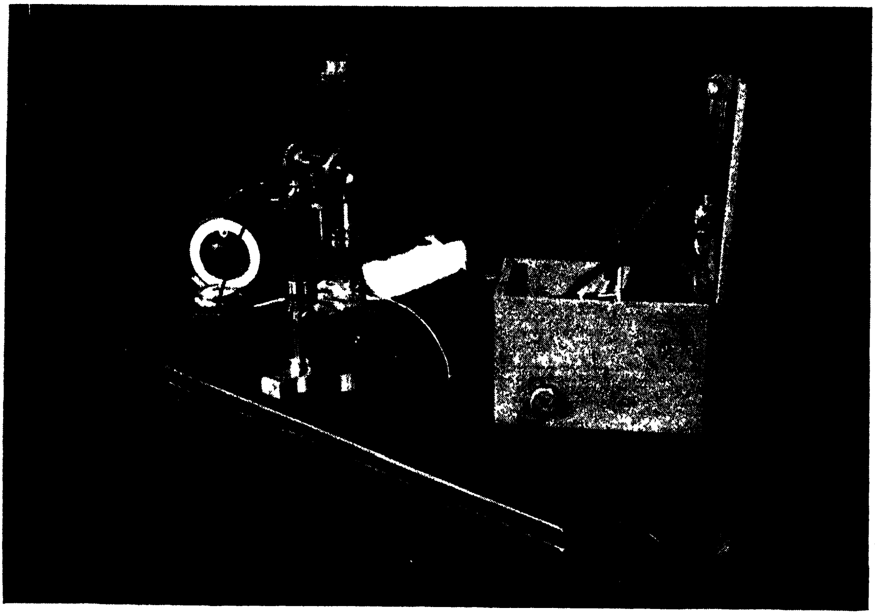

Fig. 129. Stage in Operation.

Holding Device. Originally we pinned the rat with thumb tacks to a thin animal board, put the cuff around the thigh, supported the chosen foot in a cradle of plasticine and moved the board around on the stage of the microscope by hand. In our present technic we remove the stage of the microscope and insert an animal board attached to a mechanical stage devised by Mr. Arthur Rawson and Dr. Jeffers. It is illustrated in Figure 128 and shown in

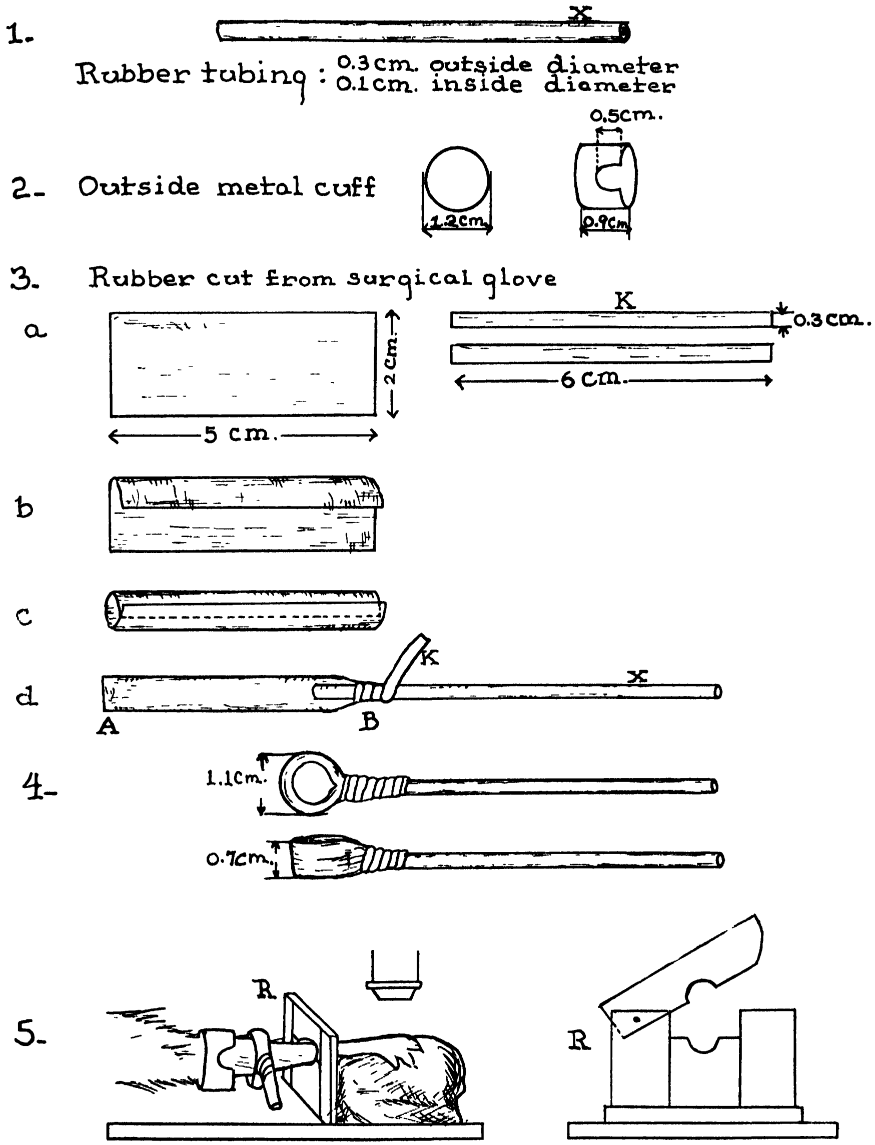

Fig. 130. Material and Procedure for Making Cuff.

operation in Figure 129. Thumb screws permit motion in two directions. The leg-holder attached to the stage is intended to encircle the thigh (when the handle is lowered) below the cuff. (See Fig. 129.) The foot rests upon a block of plasticine below the holder while plasticine covers the toes to hold the foot in place.

The Cuff. (Fig. 130.) A sheet of glove rubber 2 x 5 cm. is folded to form a tube 5 cm. long. The edges after folding are sealed with any convenient tire cement (we use cold-patching cement, made by Goodyear). The small rubber tube shown (1) is thrust into one end of the tube just described for a distance of about 7 mm. and is firmly bound into place by several turns of a narrow rubber strip K, each turn being moistened with cement. This maneuver is shown in 3, d. At the end of the tube marked A, the inside of the tube is moistened with cement and closed by pressing together. Then end A is carried over to B where they are bound together with more turns of rubber strip moistened with cement. The cuff should now be air tight. In use we fit it inside a metal cuff as shown (2) which has a cut out for the rubber tube of the cuff. An outer cuff of adhesive may be substituted for the metal cuff but is less satisfactory. The cuff, if kept dry and powdered, will last indefinitely.

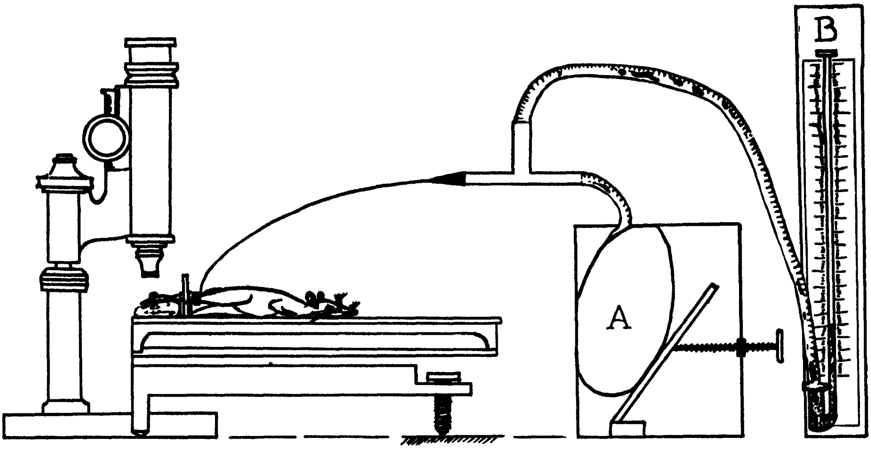

Fig. 131. Procedure for Determining Blood Pressure.

Actual Determination. The animal is anesthetized. The dorsum of the foot to be used is shaved, using a sharp blade. A depilatory, barium sulphide, may be used but is undesirable unless the animal is being prepared several days in advance, for enough edema may appear in a few minutes to interfere with capillary flow. A bit of paper is wrapped once or twice around the foot to prevent the rubber cuff tearing on the toe nails. The cuff (metal followed by rubber or both together) is then applied over the foot and pushed up to the thigh. The paper is discarded. The rubber cuff is properly fitted inside the metal cuff. The metal leg holder attached to the stage is then lowered over the thigh below the cuff. The foot is pulled down on the plasticine block and is fixed by embedding the toes in plasticine. Cedar oil is placed on the shaven area. The cuff is connected with a sphygmomanometer as illustrated in Figure 131, and observation through the microscope is begun. We use a 10 mm. ocular and 16 mm. objective (Ultropak objective 16.2). One or more vessels with rapid flow are usually easily located. Bulb A (Fig. 131) is then compressed with the thumb screw until the flow stops, and the height of the mercury column in B is noted. Several readings are usually made using different vessels, with a customary variation of 2 to 5 mm. Each determination takes about a minute. If there is a definite variation in different vessels the highest reading should be accepted, as occasionally a localized vascular spasm may make a reading too low.

Normal figures vary somewhat with the type of anesthetic, averaging about 20 mm. higher with ether than with Nembutal. The normal range with Nembutal is 60 to 120 mm. with the average about 90; with ether 80 to 140, averaging about 110. Large, old animals have slightly higher pressures than young ones. Pathologically, as after intracisternal kaolin injection, carotid sinus denervation, subtotal nephrectomy, intraperitoneal fluid injection, pressures of 150 to 300 mm. or more have been observed.

Indirect Method Using the Tail. This method as reported by F. B. Byrom and C. Wilson[2] requires anesthesia. A method similar in principle has been recently reported by Williams, Harrison and Grollman.[3] The circulation through the tail is arrested by suddenly inflating a cuff on the root to a pressure well above the systolic level. The pressure is then gradually reduced. When the systolic level is reached, blood can enter but cannot leave the tail. The resulting increase in volume of the tail is detected by a simple water plethysmograph. The method of Williams et al. (1939) uses a less sensitive plethysmograph and does not require anesthesia. The animal is warmed before and during each determination and a preliminary training period is required.

Direct Method. This is in principle the standard laboratory procedure used for larger animals. Modifications are necessary because (1) the arteries of the limbs are not available for cannulization or needling because of their size; (2) the clotting power of the rat's blood makes repeated observations impossible unless an anticoagulant has been previously added to the blood stream.

In practice, therefore, the arteries available are the two common carotids and the abdominal aorta. Blood pressure readings from the carotids are open to the objection that they may be influenced by mechanical trauma to the carotid sinus. On the other hand, the rat ordinarily bleeds to death after a needle is removed from the abdominal aorta. The procedure described is intended for observations using the abdominal aorta.

Under any desired anesthesia a long abdominal incision is made and the abdominal aorta exposed from the bifurcation of the common iliacs to the point of origin of the renal vessels. Heparin, 1.5 mg. per 100 Gm. of body weight, is then given intravenously. The heparin solution we use contains 3 mg. of heparin per cc. of distilled water. One of the veins of the abdominal wall is usually available for the injection, or one of the femoral veins may be used. A Wassermann needle is selected of nearly the same diameter as the aorta. It is connected through a tube filled with physiologic saline to a mercury manometer. There must be no air bubbles in the tubing. The mercury column should be narrow, approximately 1.5 mm. in diameter, so that movement of the column after equilibrium is reached will not result in much loss of blood from the rat. Five minutes after the heparin injection the Wassermann needle is inserted into the abdominal aorta, pointing toward the head. Blood pressure is read directly on the mercury column and changes may be followed for 30 to 40 minutes. If a single reading only is desired, the heparin may be omitted.

Blood Pressure Recording by the Hamilton Manometer.[4] This apparatus will not be described in detail. It offers a means of recording optically the intra-arterial pressure, both systolic and diastolic, using a tight manometer system to prevent coagulation of blood in it. The deflections of a mirror mounted on a silver membrane are recorded by a film travelling 3 mm. per second.

The actual procedure is as follows: Under Nembutal (or some other) anesthesia the rat is pinned supine to a board. An incision 2 cm. long is made over the femoral artery in the groin. A drop or two of 1% procaine is applied before the artery is dissected free for 1.5 cm. and another drop is applied after the dissection is completed. This prevents the artery from constricting as the result of local trauma, which would make the introduction of a needle virtually impossible. The distal portion of the artery is then ligated and a light clamp, guarded by rubber, is applied centrally. Through a small transverse incision in the artery a 20-gauge needle attached to the manometer is introduced and fastened in place by a ligature around the artery. As the central clamp is removed, citrate solution is momentarily flushed through the system, after which the manometer will record for an hour or more if the animal does not move.

Criticism of the Blood Pressure Methods. The method of Hamilton is probably the best but by far the most expensive. It is the only method that measures diastolic pressure as well as systolic. After a single determination the artery must be ligated. Hence it is unusual to be able to make more than two determinations on the same animal.

The tail method of Byrom and Wilson requires relatively inexpensive apparatus and is excellent in principle. It may be used repeatedly on the same animal. Although the authors use anesthesia the method has been used without anesthesia with trained, warmed animals. The warming, however, which produces a peripheral dilatation, may be objectionable in the study of certain forms of hypertension. The method has not as yet been used in the study of various forms of experimental hypertension, or at least has not been reported. On theoretical grounds it should work satisfactorily in cases of hypertension with elevation of both systolic and diastolic pressures. It might be less satisfactory in a hypertension characterized by an elevated systolic pressure with a nearly normal diastolic pressure. In such cases mean pressure is only slightly elevated, and the systolic peak of the pulse wave is relatively sharp. This type of hypertension is seen following the intracisternal injection of kaolin, as will be described later. In such animals we have observed flow in the capillaries of the feet while the pressure in the cuff about the thigh is raised and lowered. It has been repeatedly observed that flow may stop, while pressure is being raised, at a level 30-40 mm. above the point at which it begins again when pressure is lowered. The inertia of blood cells which have stopped moving is probably sufficient to require a little more pressure to start them moving than to keep them moving when once started. This difference is inconsequential in the normal animal but may be sufficiently marked to lead to serious error in the experimental hypertension just described. The method of Byrom and Wilson depends upon the return of flow which has been arrested.

The indirect method of Griffith requires relatively inexpensive apparatus if the worker will put up with a make-shift light and stage. Even with the conveniences described the apparatus can be assembled for about $75.00. It requires considerable training for the microscopist. The animal must be anesthetized. Measurements may be repeated indefinitely. It is poorly adapted to conditions with intense peripheral vascular spasm, for example, after the intravenous injection of adrenalin. Local vessel spasm may occasionally make results obtained too low, though this is probably unusual. It can be used in the study of all forms of hypertension so far described. The tendency to error is to make the reading too low—a consolation in work on hypertension.

"CAPILLARY" OR MINUTE VESSEL PRESSURE

By E. Roberts

We use the method of Danzer-Hooker, with certain modifications. The dorsum of the foot must be prepared several days in advance, either by depilatory or by shaving, so that all local reaction has subsided by the time of the observation. The rat, anesthetized, is placed on the mechanical stage described under Blood Pressure; the foot is placed on a block of plasticine and fixed by placing plasticine over the toes. The chamber of the Danzer-Hooker apparatus is then lowered so that the membrane touches the dorsum of the foot. We use Cellophane instead of goldbeater's skin and fill the entire system with mineral oil, which prevents the appearance of annoying bubbles when the reading is being obtained. The schema is shown diagrammatically in Figure 132. The minute vessels are then visualized through the microscope, using the arrangement described under the Indirect Method of Obtaining Blood Pressure. A spot must be selected where the Cellophane is in contact with the skin. Not all these vessels are capillaries in the histologic sense, so for this study the term "minute vessel pressure" should be substituted for capillary pressure. The course traversed by the image reaching the eye is as follows: cedar oil, Cellophane, mineral oil, glass plate, air, microscope, air, eye. The Cellophane membrane is kept in mineral oil when not in use. Vessels are selected in which flow is visible. Pressure is raised until flow stops. At this point, ordinarily but not always, the vessels disappear. This point is considered to be minute vessel pressure. It is normally 12 to 21 mm. of mercury.

Venous Pressure. The device by which venous pressure is usually determined clinically is the following: A Wassermann needle is connected to a burette or vertical glass tube with rubber tubing. The whole system is filled with physiologic saline. The needle is then introduced into a vein and the saline permitted to flow into the vein as long as it will. When equilibrium is reached, the height of the saline above the vein level equals the venous pressure. This method requires the introduction of several cubic centimeters of fluid intravenously, a procedure which is unobjectionable in a man or large animal. It is, however, inappropriate in the rat, especially when the reading is to be made in a small vein, as a mesenteric vein.

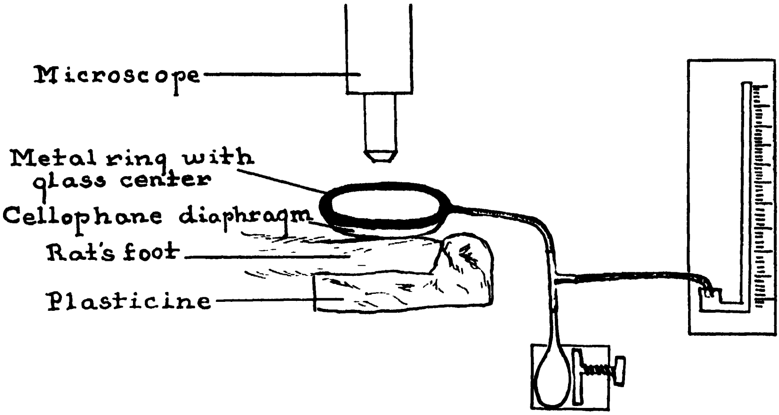

Fig. 132. Schema for Determination of Minute-Vessel Pressure.

Direct Method for Obtaining Venous Pressure in a Mesenteric Vein. The set up requires a micromanipulator. (See photograph, Fig. 133.) The one we use was made in the shop of the Medical School, University of Pennsylvania, but any standard micromanipulator may be used. A 27-gauge hypodermic needle, three quarters of an inch long, is attached to a glass tube with sealing wax or De Khotinsky cement. The glass tube is held in the micromanipulator, which must have 3-dimensional mobility. A rubber tube passes from the glass tube to a T-tube, through which it is connected with a mounted tuberculin syringe and a manometer. The manometer is simply a capillary tube mounted vertically against a millimeter scale. In actual practice a reservoir is connected to the system through a 2-way stopcock, from which the syringe can be filled with fluid at any time. This reservoir plays no part in the actual determination and, for simplicity, is not shown.

Before starting an experiment the system is filled with distilled water strongly colored with methylene blue. The manometer, completely filled, drains through the needle held in the micromanipulator until equilibrium is reached. This is the zero reading. The rat, anesthetized, is placed on a suitable stand. A loop of intestine is placed over a little block and fixed with

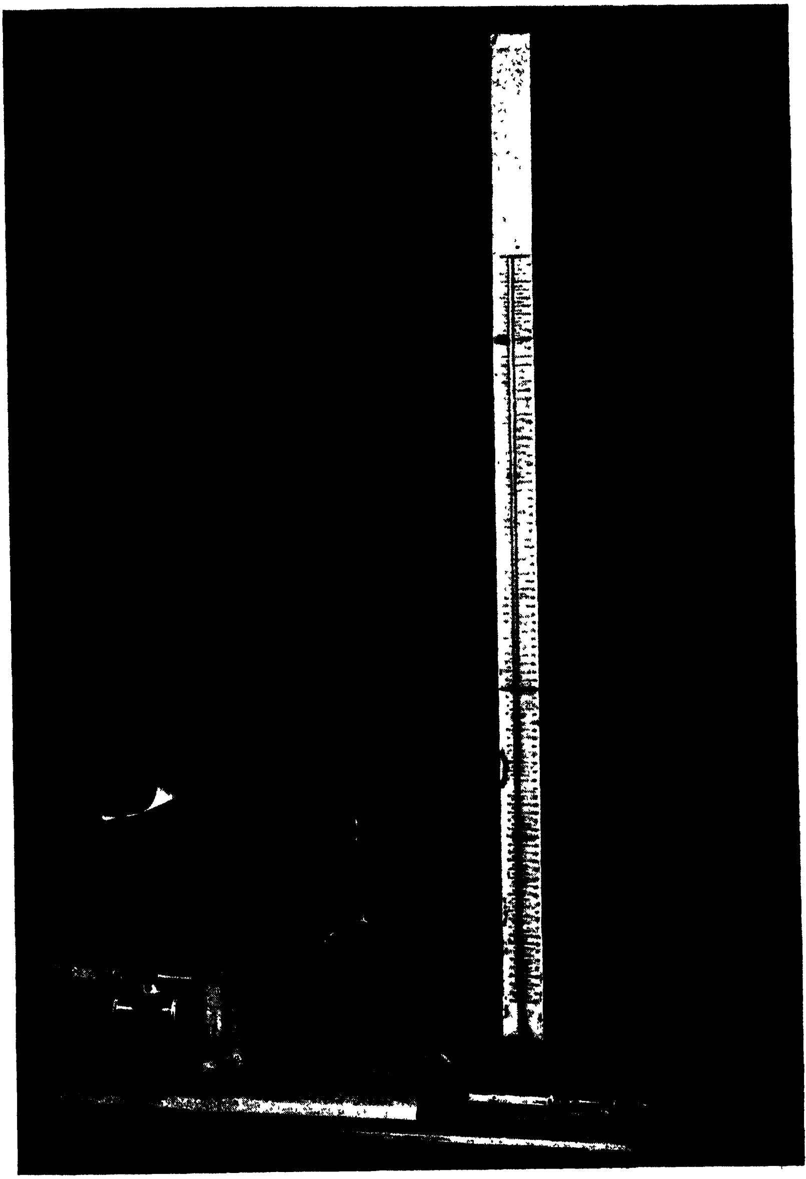

Fig. 133. Apparatus for Direct Method of Obtaining Venous Pressure.

thumb tacks. The mesenteric artery and vein are plainly visible. The micromanipulator is then arranged so that by using the sidewise and thrusting motion the needle may be expected to enter the vein. If the up and down adjustment must be used a second zero reading is taken. The vein is then observed through a dissecting microscope and, using the micromanipulator, the needle is made to enter the vein. With a little practice this can be accomplished in two out of three attempts. The piston of the mounted syringe is then compressed by an assistant, using the screw adjustment, while the operator watches the vein through the microscope. When the first blue fluid enters the vein the reading on the manometer is taken. This, less the zero reading, is the venous pressure. Normal readings are about 9 to 16 cm. of water.

METHODS FOR OBTAINING BLOOD

From the Tail. This is satisfactory when small amounts are required, i.e., from 1 to 1.5 cc. An anesthetic need not be used, but the rat may be rolled in a towel as described on page 20. The tail is lightly shaved to assist in visualizing the veins, of which there are three. It is then immersed in water at 45°C for one minute. The vein is then punctured by a cutting-edge skin-needle and the blood collected. Ordinarily, bleeding is free. When no more is desired, pressure is made over the bleeding point for a minute with a piece of gauze, and the hole is sealed with a drop of collodion. This may be repeated many times.

From the Aorta Under Anesthesia. This is a quick way to obtain 3 to 5 cc. but kills the animal. An intravenous needle attached to a syringe is thrust into the abdominal aorta, which has been exposed by a suitable incision (see p. 47). The needle points toward the head. The syringe rapidly fills with blood.

From the Jugular Vein Under Anesthesia. This also kills the animal. Both jugular veins are exposed, and one is tied off. The wound surrounding the other vein is liberally smeared with petrolatum. The vein is then cut through and held over a beaker in which the blood is collected. Clots may be washed away or their formation prevented with citrate solution. It is possible to obtain 4 to 7 cc. of blood in this way, of course diluted with citrate solution. The amount of citrate added may be calculated roughly by measuring the amount left and subtracting this from the original amount.

By Cardiac Puncture. See description under Hematology, Chapter 14, page 417.

METHODS FOR INTRAVENOUS INJECTION

Into the Jugular Vein. Anesthesia is required. In our opinion, this is the only method appropriate when small amounts must be injected quantitatively, as for blood-volume measurements. The jugular vein is exposed. Using a 27-gauge needle, ⅝ inch long, the vein is entered just above the clavicle, the needle pointing toward the animal's head. By passing through the pectoral muscle at its edge bleeding is prevented. At the time of entering the vein the needle just grazes the upper edge of the clavicle and is pointed directly backward. The direction of the needle is then changed to point toward the head. By moving the needle forward and backward it can be shown to lie free of both the anterior and posterior vein wall. Because the needle is so near the heart injection should be slow, not over 0.5 cc. per minute. Amounts of 1 cc. or more should be warmed before injection.

Into the Tail Vein. Anesthesia is not required. The animal is wrapped in a towel as described on page 20. The same size needle is used as for the jugular vein. The tail is shaved to reveal the vein more easily. A rubber-band tourniquet is placed about the base of the tail. The tail is drawn through a metal hook or loop which is fixed to a table or animal board, so that traction on the tail will hold it taut and fairly immobile. The operator holds the tip of the tail in one hand and the syringe in the other. The needle is made to pierce the skin with the point of the needle down in the usual manner. It is then rotated through 180° and inserted into the vein with the point of the bevel up. This is necessary because the vein is more intimately connected with the skin than with the underlying tissue and, if entered in the usual way, the needle will simply pass through it. Only about 30% of attempts are successful.

Into the Femoral Vein. General or local anesthesia may be used. If local is used, the rat is wrapped in a towel with one leg, as well as the tail, out. A safety pin may connect the edges of the towel between the legs for greater security. The skin over the vein and the dorsum and palm of the foot is infiltrated with procaine. The leg is then drawn through a hook or metal loop fixed to an animal board, the foot fixed by a thumb tack passed through the anesthetized area, and the leg supported by a block of plasticine. The vein is exposed by an incision parallel to its course. A rubber-band tourniquet makes it easier to enter the vein. The point of the bevel of the needle is down throughout. The vein is then entered, the tourniquet removed, and the injection given. It is best to depend upon vision to detect the moment when the vein is entered, not upon withdrawing blood into the syringe. The wound should be stitched before the effect of the local anesthesia wears off. Successful injections have been obtained in almost every attempt. From 5 to 6 cc. may be given at a rate of not over 1 cc. in 2 minutes. This is the preferred route for giving large quantities of fluid intravenously.

If the rat is given a general anesthesia, the procedure is essentially similar except that the steps for holding the animal and leg are unnecessary. The vein can usually be readily entered high up in the thigh without even the help of a tourniquet, and a somewhat larger needle may be used.

METHOD FOR DETERMINING BLOOD VOLUME

This was reported by Griffith and Campbell.[5] Nembutal anesthesia is used. As soon as the animal is sufficiently quiet, the jugular vein is exposed at the base of the neck by an incision parallel to its course. Then 0.3 cc. of 5% vital red is injected into the dilated bulb, following the technic described on page 284.

After 3½ minutes the tail is immersed in water at approximately 45°C. for one minute. Then, 4½ minutes from the time of the injection, a vein in the tail is punctured, and 50 cu. mm. of blood is withdrawn into a graduated pipette. The puncture wound is sealed with collodion. The 50 cu. mm. of blood are placed in 2.95 cc. of physiologic saline (3 cc. from which 50 cu. mm. have been removed) and centrifuged. The supernatant fluid is pink.

The standard is prepared by adding 0.3 cc. of 5% vital red to 4 cc. of physiologic saline, the resulting volume being 4.3 cc. This is the primary standard. Standard A is made by adding 50 cu. mm. of this to 5.95 cc. of physiologic saline (6 cc. from which 50 cu. mm. have been removed). Standard B is made by adding 100 cu. mm. of the primary standard to 5.9 cc. of physiologic saline.

We use a microcolorimeter* requiring 2 cc. of fluid in each cup. The standard cup is connected by a sidearm to a 1-cc. tuberculin syringe graduated to 0.01 cc. The volume of the standard in the standard cup (VS) is therefore 2.00 minus the amount in the syringe, read to the second decimal. In an actual determination 2 cc. of the unknown are introduced into the cup for the unknown. This is the pink supernatant fluid obtained after centrifuging. Two cc. of standard A or B are introduced into the standard cup, selecting the standard which appears slightly darker than the unknown. Standard is then withdrawn into the syringe until the colors match. The formulas are: for standard A, blood volume = 17.2/VS; for standard B, blood volume = 8.6/VS.

The average blood volume by this method is 4.3 cc. per 100 Gm. of body weight, varying from 4.0 to 5.3 cc. The smaller animals have the larger blood volumes in terms of body weight.

If the course of disappearance of the dye is followed at 15-minute intervals after injection, it is found that a gradual loss occurs reaching 11 to 12% in one hour.

Method for Repeated Determinations

This method is applicable when dye from a previous injection is still present in the blood stream. The only change is in the making up of standards A and B. It is estimated from the size of the animal which standard, A or B, will be required. If there is doubt, both must be made up. Take 2.95 cc. of the selected standard and add to it 50 cu. mm. of blood taken just before the second dye injection. Centrifuge, and place 2 cc. of the supernatant fluid in the standard cup. The remainder of the procedure and calculation is unchanged.

PATHOLOGIC STATES ARISING SPONTANEOUSLY

In measuring the blood pressure of hundreds of rats of various ages we have noted a tendency to a slight rise with age. Whereas rats of weight less than 100 Gm. will seldom have a systolic pressure over 100 mm. of mercury, old rats weighing 300-400 Gm. may have pressures around 150, rarely 160. We have seen two old males with pressures around 190. On this account we have tried to exclude from our experimental work all animals over 250 Gm.

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* The colorimeter used is the Vim-Sheftel Microcolorimeter, made by the MacGregor Instrument Co., Needham, Mass.

Wilens and Sproul[6] have examined necropsy material of 487 albino rats maintained on adequate diets and permitted to die spontaneously. Over half the animals presented fibrosis of the myocardium and sclerosis of the coronary arteries and about a third showed some hypertrophy of the myocardium. Cardiac lesions seen less frequently included intracardiac thrombosis, chronic auriculitis, acute bacterial endocarditis, "chronic endocarditis" without valvular insufficiency, calcification of the coronary arteries, abscess of the myocardium, interstitial myocarditis, acute suppurative pericarditis and chronic pericarditis. In the vascular system they found that the elastic fibers in the aortas of senile rats were thicker and less undulating than those of young ones. Intimal thickening did not occur. Degenerative changes were found, only in the coronary and pulmonary arteries, consisting of fibrosis of the media and thickening of the wall. Calcification was frequently found in the pulmonary arteries, occasionally in other arteries, especially the spermatic. A specific inflammatory disease of arteries identical with or at least closely resembling periarteritis nodosa in man was found in 9.7% of the animals. Renal lesions similar to arteriosclerotic atrophy in man were described but their association with vascular disease could not be established. The arterioles showed evidence of sclerotic change in only a few exceptional cases and in none was generalized arteriolosclerosis recognized.

INDUCED PATHOLOGIC STATES

Experimental arteriosclerosis in the rat has been produced by Sweeney and Smith[7] by feeding 50 cc. of viosterol to each animal over a period of 25 days. This was proved by x-ray examination of the aorta and by histologic section. Blood-pressure measurements were not made.

Methods for Producing Hypertension

Intracisternal Kaolin Injection. Cisternal puncture is performed as described on page 192. As much cisternal fluid is removed as will come easily; this is never over 0.05 cc. Slightly less of kaolin suspension is injected. The kaolin we have used is Crooke's Collosal Kaolin. Approximately 30% by weight is boiled in distilled water and cooled to about 37°C. for use. Following a successful injection, a vascular hypertension will usually appear in 5 to 8 days.

Subtotal Nephrectomy. This is the method of Chanutin and Ferris.[8] Hypertension may develop in 1 to 2 weeks. In our hands the mortality has been high. We secured 6 hypertensive animals out of the first 100 operated. Subsequently we have had much better success and a lower mortality by using the following modification: (1) Only one ligature is placed about the upper pole of the kidney which is to remain. Thus about half of a kidney is left instead of one-third; (2) We give 5 cc. of physiologic saline daily intraperitoneally for a week after the second operation (the nephrectomy).

Carotid Denervation. For details of technic see page 441. A unilateral denervation usually leads to an immediate rise in blood pressure, which usually returns to normal by the next day. A bilateral denervation has never led in our experiments to a hypertension lasting more than 3 days. In the rat the carotid nerve cannot be seen. Its section is a matter of chance in the dissection. Sometimes, judging by the blood-pressure response, it is separated by merely strong traction on the vessels; again it appears to escape the most mutilating dissection.

By Giving Extravascular Fluids. This is the method reported by Griffith, Jeffers, and Lindauer in 1937.[9] Forty % of rats injected with physiologic saline or distilled water, either intraperitoneally or subcutaneously, in amounts of 15 cc. per 100 Gm. body weight, will develop a hypertension in 24 hours. This has continued 24 to 48 hours. The mortality is less with saline, which is therefore preferable.

Constriction of Renal Artery by Modified Goldblatt Clamp. The little screw clamp of stainless steel (see Fig. 128) was designed and made by Mr. A. J. Rawson of the Johnson Foundation, University of Pennsylvania. This size is suitable for use in rats weighing 150-250 grams. Under light anesthesia the kidney is exposed through a dorsal, retroperitoneal, muscle-splitting approach.

It is left in place while the renal artery is dissected free for a distance of 5 mm. from its origin at the aorta. The female portion of the clamp is placed around the artery by means of a hemostat, and the screw is slowly tightened until the clamp will barely slide along the artery under firm pressure. The other kidney is removed completely a week later. This degree of constriction will, in about 20% of rats, produce hypertension without renal infarction.

Drury's Method for Rabbits. This method almost certainly could be adapted for rats. He places a loose silk ligature about the left renal artery of a baby animal, and allows this loop to serve as a check to normal kidney growth

THE LYMPHATIC SYSTEM

By J. Q. Griffith, Jr.

The lymphatic system includes both the lymph nodes and the lymphatic channels. Fluid passes from the intercellular spaces into the lymphatic channels which unite into successively larger channels. At intervals the fluid in them passes through lymph nodes, which serve to remove certain harmful substances. Eventually the lymph returns to the venous system through the thoracic duct or similar vessel. Changes in rate or volume of lymph flow may depend upon: (1) Changes in the rate and volume of lymph formation; (2) Pathologic changes in the lymph channels and lymph nodes. Lymph formation is increased in conditions that produce hyperemia, as in exercise of a part or partial obstruction of its vein. Lymphatic channels may be partially blocked as the result of infection, by the previous injection of an irritating substance such as kaolin, by the infiltration of malignant tumors, or by external pressure. If simply transected, by the cut of a knife they rapidly reform. Lymphatic channels below the point of obstruction are usually dilated, and general edema is the rule.

While studies of the lymphatic system might theoretically be carried out by various methods, in actual practice there are primarily four:

1. Direct cannulization of a lymphatic by a fine tube and collection of lymph. This has been done in man and dogs by C. K. Drinker and his colleagues.[11] Probably it has not been done in the rat.
2. Direct visualization of lymphatic vessels of the mesentery of the living rat by the method of Webb.[12] The lymphatic vessels accompany the mesenteric blood vessels, and, under the microscope, segmental contractions between valves were observed at the rate of 12-18 per minute. For best results the rats should be fasted to dispose of mesenteric fat.
3. Intracutaneous injection of highly colored colloid dyes which are carried away in the lymphatic system, giving rise to streamers.
4. Injection of substances which enter the lymphatics and later can be detected in the lymph nodes. A great variety of substances, such as ink, pigeon's corpuscles, and thorotrast, may be used if the node is to be sectioned and examined histologically. Such studies are laborious, requiring serial sections of an entire node if quantitative information is to be secured. Thorotrast alone can be detected in roentgenograms of the node made at varying times after the injection.

Lymphatics of the Skin

The dye used has been Patent Blue V, prepared and used according to the method of McMaster.[13] The details of preparation will not be given because the dye is no longer obtainable commercially, and should be supplanted by Evans Blue, the dye now widely used in the determination of blood volume.

Either 0.01 or 0.02 cc. of the dye is injected into the skin as superficially as possible. Either amount may be chosen, but once chosen should not be varied. The blue area is traced on Cellophane with ink. The Cellophane should not be water proof or it will not take the ink. However, water-proof Cellophane may be used if its surface is first lightly rubbed with a sponge soaked with ether. A new tracing is made every 5 minutes for half an hour. The entire set of tracings are labelled and can best be preserved by clipping on a piece of white paper. Each observer must make his own set of normal standards, allowing for the area injected and his particular manner of injection. In general, it will be found that lymphatic flow is faster (i.e. the streamers extend more rapidly) in the limbs than on the trunk. Running movements will increase the length of the streamers. So will a low plasma protein, or a previous gavage of water, 5% of the body weight, by stomach tube. In areas of edema the streamers are short but very wide.

Visualization of the Lymph Nodes With Thorotrast

The relative frequence of calcium deposition in the lymph nodes of the rat makes a preliminary roentgenogram essential. Subsequent roentgenograms, obtained after the injection of thorotrast, must be compared with this one.

Thorotrast may be injected anywhere, into any tissue, and subsequent roentgenograms will show the lymph nodes draining the area which was injected. The time interval varies with different locations. For example, after injecting thorotrast into the dorsum of the foot the lymph node near the knee will appear in 10-30 minutes (Chapter 15, Figure 145). After injection into the cisterna magna the cervical lymph nodes will appear in 30-45 minutes. However, after injection into the anterior chamber of the eye, without leakage of thorotrast, no lymph nodes will be visible in 10 days. In general, the periphery of the node is seen first, giving the appearance of a ring the center of which soon becomes opaque.

As a rule normal lymphatic channels are not shown by thorotrast, with the possible exception of the perineural spaces of the brain which, functionally at least, correspond to lymphatic channels (see Chapter 15, Figure 148).

METHOD FOR PRODUCING CHRONIC LYMPHEDEMA

A suspension of kaolin 30% by weight in distilled water is used. This is injected in a ring completely encircling the ankle of a rat. About 0.5 cc. is required. In 2-3 weeks chronic lymphedema of the foot will develop, usually with an area of ulceration. The point of obstruction is probably fairly high, at least above the knee. If thorotrast be injected into the foot the dilated lymphatic channels will show in the roentgenogram (see Chapter 15, Figure 146). The condition appears to be permanent. Undoubtedly other substances might be used, but kaolin has been quite satisfactory. Drinker in dogs has used a suspension of finely divided crystalline silica in 2.5% quinine hydrochloride.

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12

The Use of the Rat in the Biologic Assay of Hormones

By

DWIGHT J. INGLE

IN THIS chapter are described methods for the use of the rat as a test object for the biologic assay of estrogenic hormones, the life-maintaining hormone of the adrenal cortex, the growth and the adrenotropic hormones of the anterior pituitary, and of the gonadotropic hormones. For the biologic assay of other hormones or hormone-like substances the methods most generally accepted require other animals as test objects.

PRINCIPLES OF BIOLOGIC ASSAY

In biologic assays the experimenter measures the amount of a substance or a mixture of substances required to induce a specific biologic response in an animal organism. The same biologic response may be elicited by different chemical substances. Biologic tests may therefore be used to identify a pharmacologic property of the material tested but do not provide proof of the chemical identity of the substances. The test object for a hormone should be either an animal from which the endocrine organ which secretes the hormone has been removed, or it should be an animal in which that organ is not actively secreting the hormone at the time the test is conducted. The assay test is then based upon a biologic response which occurs normally when the gland is present and functionally active.

The quantitative relationship between the amount of a hormone administered to an animal and the response produced varies among different animals and in the same animal at different times. Possible causes of this variability include: the rate at which the hormone enters the body fluids of the animal, differences in the concentration of substances other than the hormone which are present in the solvent containing the hormone, the nature of the solvent, seasonal factors (temperature, humidity, light, etc.), diet, sex, age, size, state of health, increased sensitivity or increased resistance to the hormone following repeated exposure of the animal to it, and inherited individual differences.

A hormone is usually more efficiently utilized when its entrance into the body fluids is slow and prolonged rather than when absorbed rapidly but intermittently. The route of administration (intravenous, intraperitoneal, intramuscular, subcutaneous, intradermal, percutaneous, or peroral) alters the rate of absorption and consequently the duration of the effect of the hormone. The nature of the solvent affects the rate of absorption. The presence of other substances in the solution may affect the rate of absorption and may either augment or mask the biologic effect of the hormone.

It is probable that most hormones are secreted continuously but at a variable rate and that they enter the blood stream in amounts which are compatible with optimal utilization. The amounts of hormones which are secreted into the blood stream to maintain their normal physiologic functions are not known. In any practical method for administering hormones to animals for the purpose of biologic assay the entrance of the substances into the body is intermittent rather than continuous, and undetermined amounts of these substances are probably wasted by the body. Differences in the apparent activity of the different preparations may be due to differences in the efficiency of utilization as well as to differences in content of hormone.

Even with all procedures carefully controlled, individual differences among the test animals will affect the response to equal amounts of hormone. The experimenter must determine the range and frequency distribution of the biologic responses to take proper account of the error of sampling a population of test animals. Statistical procedure is recommended. He must determine how many animals must be used in an assay test to detect reliably a given difference in the concentration of two samples of a standard preparation of a hormone. To obtain an answer to this question the following steps for the standardization of an assay method should be carried out:

1. Establish the relationship between the amount of a standard preparation of the hormone administered and the frequency or amplitude of response in a large series of test animals. From this relationship the experimenter can select that range of frequency or amplitude of response within which the curve makes its sharpest ascent and thus select a standard amount of hormone to be used in the assay.
2. The standard dose of the hormone selected in Step 1 should be administered to a large series of test animals. From the standard deviation of the distribution of responses the experimenter can calculate the minimum number of animals required to insure the desired degree of accuracy in the assay. Simple procedures for the mathematical interpretation of bio-assay results have been described.[2, 6]

The responsiveness of test animals varies from one laboratory to another. It is advisable for each laboratory to establish its own standards. Comparisons of assay values from one laboratory to another can be made by use of a common standard preparation of the hormone. The response to the preparation being assayed should, if possible, be compared to the response to a standard preparation tested at the same time.

METHODS

All of the procedures here described have been found useful for biologic assay when properly standardized, but are time consuming. More rapid and sensitive tests may be anticipated.

Estrogenic Substances

In the rat the state of estrus can be detected by the presence of cornified cells in the vaginal smear. Allen and Doisy[1] introduced a method for the quantitative assay of estrogens based upon the amount of the substance required to induce estrus in the castrated sexually mature female rat. A modification of their technic is described here.

From female rats weighing at least 140 grams, the ovaries are aseptically removed with a small portion of the upper horn of the uterus to insure complete removal of all ovarian tissue. After a minimum period of two weeks the tests are initiated. The test material is administered in three divided doses by subcutaneous injection at intervals of four hours. The solvent is physiological saline and the volume of each injection is kept constant at 0.5 cc. The vaginal smear is made by inserting a cotton swab moistened with physiological saline into the vagina, rotating it gently a few times, spreading the material on a glass slide and examining it under low power. The smear is termed positive when all of the cells present are fully cornified or when only a very few leukocytes and epithelial cells are seen in addition to the cornified cells. The first smear is made 40 hours after the completion of the injections and is repeated at intervals of eight hours until three smears have been made upon each animal.

Estrone is an accepted standard estrogenic substance. One international unit indicates the estrus producing effect of 0.0001 mg. of estrone. It may therefore be used in the standardization of the method. It has been found that the best quantitative results are likely to be obtained when the experimenter uses amounts of the test preparations required to produce estrus in approximately 50 per cent of the test animals.[3] When the incidence of estrus falls near the 50th percentile the experimenter can make use of the curve of relationship between dosage and incidence of estrus (Step 1 of the procedure recommended for standardization) for interpolation.

The test animals should not be used more frequently than once during each two-week period. An increase in sensitivity to estrogenic substances may be observed following the first few tests. It is therefore advisable to sensitize the test animals before using them for quantitative studies. The animals may be used as test objects repeatedly until they reach the age of one year or show evidence of illness.

Substances Which Will Maintain Life in Adrenalectomized Animals

The use of the rat for the assay of those substances which will maintain life in adrenalectomized animals is less desirable than the use of adrenalectomized dogs. The time for the survival of untreated adrenalectomized rats in different laboratories varies widely. Several causes of this variability have been identified. There may be strain differences in survival time which are due in part to differences in the amount of accessory cortical tissue. The age and sex of the animal, the intake of sodium and potassium and possibly other unidentified factors in diet, operative procedure, postoperative care, temperature, and unidentified seasonal factors are each capable of influencing the periods of survival and the maintenance requirements for the test substances.

A strain of rats must be used which shows a high incidence of mortality among the untreated adrenalectomized animals. Either young males or young females may be used but only animals of the same sex. The amount of potassium present in the diet should approximately equal the amount of sodium present. If it is desirable to reduce the times for survival of the untreated adrenalectomized animals this can be accomplished by increasing the potassium content of the diet and reducing the sodium content. The operations are carried out under light ether anesthesia using clean but not necessarily sterile technic. Loss of blood and unnecessary trauma to the surrounding tissues are avoided. The adrenal bodies together with the fat tissue surrounding them are removed without rupture of the capsule of the gland. If infections of the wounds develop postoperatively more rigid technics must be employed. Following operation the animals are maintained in clean, dry cages within a temperature range of 70-85°F.

The animals should be 28 days of age at the time of operation. The hormone is administered by subcutaneous injection twice daily. The volume of injections must be kept constant. The injections are continued until the death of the animal or for a period of 21 days. If the experimenter has available a sufficient amount of pure cortin-like compound such as desoxy-corticosterone it may be used as a standard. The activity is calculated from the minimum daily amount which will induce a minimal gain in body weight in a definite percentage of a group of adrenalectomized animals. The values to be used must be defined in each individual laboratory on a basis of the relationship found by that laboratory to exist between dosage and survival and rate of body growth. Comparisons of values between laboratories can be made by reference to a common standard. The maximum incidence of survivals among the untreated animals should not exceed 10 per cent and the minimum incidence of survivals for the treated animals should not be less than 80 per cent.

The procedure for the administration of cortin may be modified by placing the hormone in the drinking water. This method has the advantage of yielding a better quantitative relationship between dosage and response and of small requirements for maintenance. In the application of this procedure it is required that each animal be kept in an individual cage and that the experimenter anticipate the amount of fluid which each animal will voluntarily consume within each 24 hour period.

The Adrenocorticotropic Hormone

In the normal rat the adrenal cortices increase in size following the injection of a variety of toxic substances. This response is mediated by the anterior lobe of the pituitary. Extracts of pituitary tissue may be somewhat toxic to the test animal, therefore it is advisable to use the hypophysectomized animal as a test object for the adrenocorticotropic hormone.

Hypophysectomy is performed on male rats weighing 140-150 grams. Following the operation the animals are injected twice daily with the extract for a period of ten days. At the end of the test period the animals are etherized and exsanguinated. The adrenal glands are removed, cleared of the surrounding fat and connective tissue and weighed under cover. The weights of the adrenal glands are compared with the weights of adrenal glands of untreated animals, and with those of a series treated with a standard preparation of the hormone. The activity is calculated from the total amount of extract required daily to obtain adrenal glands of a minimal average weight. This minimal value must be established by the experimenter as described in Step 1. It should be greater than the upper limit of the range of weights in the untreated animals.

In weighing adrenal glands large errors may be induced by differences in the amount of tissue removed from the surface of the gland and by differences in the amount of moisture lost during handling. The time of exposure of the gland to the air must be minimized and kept constant. The capsule of the gland must not be ruptured. The reliability of this procedure can be tested by removing the adrenal glands from a series of rats which vary widely in body weight. Each gland is quickly excised with its surrounding fat and covered by the moist folds of intestines. When the individual glands are then presented to the experimenter as "unknowns" for weighing, the agreement between the weights of the right and left glands of the same animal provides an index as to the accuracy of the weighings.

The Gonadotropic Hormones

The methods of Fevold[5] for the biologic assay of the follicle stimulating hormone (FSH) and the luteinizing hormone (LH) are described here. Female rats 21 days of age are used for the assay of the follicle stimulating hormone. The test animals are injected for three days with two divided doses, in the morning and in the late afternoon. The necropsy is performed on the morning of the fourth day, and the index of hormonal activity is the weight of the ovaries. One rat unit of FSH is that amount of hormone which produces an average increase in the weight of the ovary of 50-100 per cent over the average weight of the ovaries of the uninjected controls. The gain of weight of the ovaries is influenced by the presence of LH in the extract containing FSH. For the qualitative detection of LH the use of hypophysectomized female rats 28 days of age is recommended. If large doses of the extract are injected twice daily for five days without producing luteinization of the follicles it may be considered to be free from LH. In the assay for FSH it is essential that the solution be free of LH, otherwise false values for FSH activity will be obtained because of the synergistic effect of combining the two principles.

For the standardization of the luteinizing hormone two units of FSH are combined with each test dose of LH. The test dose is then divided into ten individual doses and injected twice daily over a period of five days. One rat unit of LH is the total amount required to produce a 100 per cent increase in the average weight of the ovaries over the average increase produced by the administration of FSH alone.

The Growth Hormone of the Anterior Pituitary

The method described here for the assay of the growth hormone is a modification of the procedure of Evans and Simpson.[1] The adult female rat which has reached a plateau in normal body growth is used as a test object. A plateau is defined as a gain not greater than ten grams in body weight during a period of ten days. The age of the test animal should be between five and eleven months. The extract is administered by intraperitoneal injection twice daily for a period of 20 days. The standard requirement for a weight gain must be based upon a study of the relationship between weight gains and dosage. Evans and Simpson[4] have recommended the requirement of a minimum average gain of 50 grams in 20 days.

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13

Dosage of Drugs for Rats

By

HARALD G. O. HOLCK*

IN REVISING this chapter the author has attempted to include dosages and pertinent information concerning the large number of substances developed during and after World War II, as well as to add or substitute data in regard to many of the older ones. Because of the overwhelming number of articles dealing with the rat and because of the limited amount of space and time available, it has been necessary to confine the information to that selected from a limited number of journals of pharmacology, mainly in the English language. A considerable rearrangement of the material has become necessary in order to reflect, as far as possible, recent changes in nomenclature. Moreover, in order to save space, omission has been made of the often lengthy chemical names of official or well-known substances. In order not to make the chapter too unwieldy, it was further decided to delete 59 drugs and poisons from the list given in the first edition; hence information about dosages of the following substances will be found in that edition only:

Acriflavin; Amine, Dibutyl; Amine, Diethyl Ammonium; Amine Ethanol; Amine, Ethyl Ammonium; Amine, Methyl Ammonium; Androstanedione (3,17);Δ5-Androstenediol-3 (α)-17(trans); Anhaline; Benzothiazole; Cephaeline; Chaulphosphate; Conium; Cyanogen Iodide; Cytisin Nitrate; Dendrobine; Diamine, Diethyl-paraphenylene; Diamine, Dimethyl-paraphenylene; Diamine, Ethylene; Diazoiminobenzene; Dinitrophenylmorphine; Erythrosin; Estradiol-17-monopropionate; Ferric Acetate; Ferrous Citrate; Fuorate, Amyl; Germanium Dioxide; Guaiacol; Harmine; Helleborus Niger; Hexosediphosphate Sodium; Hordenine; Hydrastinine; Kojic Acid; Lead, Triethyl Salicylate; Methylene Green; Mistletoe; Neodynium; Neoprontosil; Neuronal; Phloroglucin; Piperidine; Piperonal; Proponal; Psicaine; Pyrocatechin; Ricin; Rodilone; Salicylate, Ammonium; Saligenin, Brom; Samarium; Sulfanilamide, Benzyl; Sulfonmethane; Thioamytal; Thiobarbital; Thyocyanate, Lauryl; Thiocyanate, Methyl; Thioncarbamate, Ethyl; Thiosinamine.

In regard to the factors which modify dosage the author has included a considerable number of studies, believing that a thorough knowledge and understanding of these is of great value to the beginner in pharmacology and that a fair-sized compilation of data bearing on these factors may also be useful to other workers in this field; in some cases information of this nature is found only in this part of the chapter.

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INTRODUCTION

In this chapter we have tried to collect and summarize information on the dosage and actions of drugs for the convenience and guidance of students and investigators who employ the rat as an experimental animal. For the reasons given below the tables could not be made all-inclusive. The information, necessarily abbreviated, is not of equal reliability or importance. The possible reasons for these inequalities will be discussed subsequently.

In making a selection of drugs and poisons for a dosage table many worthy contributions necessarily had to be omitted. Among the reasons for this have been that the table includes reports giving approximately the same values, that the omitted studies touched only incidentally upon dosage, that in some cases the original paper was not available for evaluation and that it was impossible to cover all of the literature within the time available.

In the practical use of this dosage compilation it must be kept in mind that values are approximate. There are various reasons for this. In the first place, different rat stocks vary markedly in resistance;[165, 405, 507, 740, 754, 769] greater resistance in wild rats has also been noted;[203] even rats in the same laboratory show unexplained differences from time to time (see Cinchophen and Neocinchophen). Secondly, it is difficult to reproduce exactly the same experimental conditions. In the third place, the end-point for the typical action of a drug is not always the same; in fact, an animal may die as a result of one effect with a small dose, while death from a different action follows a larger dose, as with scilliroside.[293] Furthermore, the term "Minimum Lethal Dose," or MLD, in the past has designated values ranging from a dose killing an occasional animal to one killing all;[398, 845] because of large individual variations, the latter two doses may differ widely. Likewise the term "Maximum Tolerated Dose" has been variously defined as one ranging from the maximum dose that would not kill any animal to one well within the fatal range. Although it is difficult and costly to determine accurately doses bordering between those tolerated by all and those killing only an occasional animal, modern mathematical treatment of experimental data obtained from a relatively small number of animals insures at least a fair approximation. To insure unequivocal terminology Foster has proposed substituting the LD 1 ("Lowest Lethal Dose") for the "Maximum Tolerated Dose" in determining the safety margin of a drug.[266] The LD 1 is defined as the dose which will kill 1% of a group of animals. It is slightly higher than the maximum tolerated dose, for the latter expression implies the highest dose with 100% survival, no matter how large a group is used. The technic of such determinations has been discussed by the same author,[266] who has based his work upon that of Bliss.[86] Sometimes the determination of the fatal dose is complicated by the fact that rats may apparently recover from a drug, only to die from secondary complications.[395, 545] Finally, medicinal products are not always uniform.

Fortunately, in recent years, uniformity has been increased by using reference standards for comparison, by adopting the term "Average Lethal Dose," or LD 50, which designates a dose that kills 50% of a group of animals, and by isolation of active principles of certain crude drugs (especially important where the components are not in constant proportion and tend to counteract one another pharmacologically, as in the case of opium or pituitary preparations). More complete discussions of these aspects may be found in such standard works as those of Burn,[119] Munch,[607] Fisher,[253] Snedecor,[781] Mainland[542] and Mather.[559]

FACTORS THAT MODIFY DOSAGE

Many of the factors which modify dosage have been subjected to renewed investigation in recent years. The discussion which follows presents briefly some of these aspects, and it is hoped that it will assist in interpreting the table and in stimulating further fundamental work of this nature upon the rat.

Temperature. The *room-temperature* at which an experiment is carried out may be of paramount importance, as in the case of insulin convulsions or acetonitrile mortalities. Very low temperatures prevented the stimulation effects of dinitrophenol[824] and necessitated more pyridoxine;[326] and the lethal dose of thyroxin for rats varied with the temperature. At 5° C. rats went into an anesthesia-hibernation condition with 5% carbon dioxide in the air, and a somewhat similar depression was seen with low and high oxygen tensions.[39] At low temperatures rats were more susceptible to paraldehyde, morphine and pentobarbital,[370] to the antipyretic action of diphenylpyrazoline carboxylic acid[722] and of quinine alkaloids,[143] and to the thyroid mitosis stimulation by thiouracil, which was practically absent at 37° C.; more thyroxin was needed to counteract the effect in the cold;[198, 640] also, at 20° C. weanling rats required less thiamine and choline for optimum growth than at 32-33°.[583] However, at low temperatures rats were more resistant to rattlesnake venom.[137] Distinct differences with certain barbiturates were noted at 20° and 30° C., respectively;[665] within certain limits, shorter sleeping times with n-propyl-o-tolylurea resulted with higher wet-bulb or dry-bulb temperatures.[189] On the other hand, within the range of about 24°-37° C., potency of certain hypnotics showed no apparent relation to temperature.[188, 822] At 27° C., rats tolerated a high oxygen concentration better than at 32°;[418] older rats were more resistant to ephedrine and vonedrine when housed at 26° than at 32° C.[889] Procaine toxicity in rats was relatively constant only between 17° and 24° C.[757] The value of the "temperature-toxicity factor" depends upon the substance and the animal under investigation.[460] To secure greater uniformity, many investigators keep rats in thermostatically controlled or in air-conditioned quarters and conduct their tests at a specified temperature.

Light. In case of photosensitizing substances, such as hematoporphyrin, eosin and hypericin,[407] a dose which was fatal in *sunlight* was safe in the dark. However, darkness lowered the resistance to uranium in guinea-pigs.[438] Also, absence of sunlight is essential in certain vitamin-D studies.

Atmospheric Conditions. Although severe reductions of *barometric pressure* definitely modify the response of animals to drugs,[558, 763, 824] the ordinary barometric variations or trends were found to be without influence upon hypnotic potency;[188] but subsequently it was concluded that duration of sleep was lengthened with increased barometric pressure.[189] Higher altitudes increased the toxicity of strychnine[597] and red squill, the latter markedly only in male rats;[884] it has subsequently been reported that female rats were more resistant to lowered barometric pressure than males.[294] Neoarsphenamine was most toxic with low temperature and high atmospheric pressure; it was more toxic when the temperature rose and the barometer fell abruptly, or when temperatures fluctuated greatly.[617] In the frog, at least, *electric disturbances,* as during thunder, markedly raised the fatal digitalis dose.[312]

With regard to *season,* better recovery from insulin in the summer has been reported for rats,[166] but higher resistance to follicular hormones has been found in the winter.[65] Puzzling is the announcement that the stimulant caffeine increased the metabolism of rats in the winter and spring, but actually lowered oxygen consumption in the summer and autumn;[382] also, that it increased the sodium excretion during the winter but decreased it in the summer.[872] Pyridine toxicity varied with the season, the toxicity being least in hot weather.[58] The duration of anesthesia after certain hypnotics was shorter in January than in April, July or October, but at several times unexplained intraseasonal high resistance was observed;[189] considerable aperiodic variation also occurred in the response to vitamin D.[431] In the studies on hypnotic power there were no significant *diurnal variations;* however, mice with low body weights showed greater sensitivity to insulin in the evening.[357] A high *humidity* gave more rapid recovery from anesthesia with certain hypnotics.[189, 278] Rats tolerated high oxygen concentrations better at 30 per cent humidity than at humidities below 20 or above 90 per cent.[418]

Emotion. As to the effects of excitement and fear, barbital-treated rats, caged with a rabbit, though not attacked by it, remained awake longer than the controls.[385] The toxicity of certain sympathomimetic amines, including epinephrine, was much greater in mice kept in groups than in those kept in individual containers.[144a]

Diet. This is extremely important in vitamin and hormone studies. Thus, for example, specific diets are required in the bio-assay of vitamins A and D and of parathormone (see Chapters 5 and 12). Special precautions are needed lest rats reingest medicines which may have been voided with the feces, such as certain estrogens;[801] likewise, one must prevent the animals from securing any material under study from the cage, such as iron or zinc. Although food has been recommended as a suitable medium for incorporation in prolonged studies,[23] such incorporation may have to be made frequently, lest aging modify the effects, as with sulfite,[272] or fermentation occur, as with glycerin.[396]

Apparently on a supposedly adequate laboratory diet, "Harvard" rats were much less resistant to thiourea than they were on Purina Fox Chow; however, recently captured wild Norway rats on the laboratory diet showed but little difference in resistance from what they showed on their normal forage.[203] Variations in fat, carbohydrate and protein proportions of growth-supporting diets did not alter the resistance to alpha-naphthylthiourea (ANTU); added cystine, with sufficient choline in the diet, increased the resistance for only a few weeks.[130] Distinct differences in hypnotic potency, in cyanide mortality and in thyroxin-stimulating effect as a consequence of varying diets have been reported.[1, 386, 573, 714] Male rats were more resistant to red squill on a high protein—low carbohydrate diet;[521] a high-protein low-fat diet made the liver more resistant to daily quinacrine doses than did a low-protein or high-protein, high-fat diet.[735] A high-protein diet reduced growth retardation and tissue injuries due to quinacrine[260] and protected rats against sulfanilamide, whereas drug intolerance was increasingly apparent on a general maintenance, a high-fat, and a high-carbohydrate diet, respectively.[436] The toxicity of cadmium and of dichloro-diphenyl-trichloroethane (DDT) were increased by low-protein diets,[255] whereas a low-fat diet increased and a high-fat diet decreased the resistance to the latter.[712] Inadequate protein intake lessened the resistance to lead chloride.[33] A high proportion of casein or methionine in the diet afforded good protection against selenium salts;[721] sulfonamides were more toxic to young rats on a purified diet with casein as the protein source than on a commercial chow, and growth, completely inhibited by sulfonamides, was strikingly increased by addition of dry, normal feces.[351] A low-protein, choline-deficient diet increased the susceptibility of young rats to dichloropropane; resistance was raised by choline plus l-cystine or by dl-methionine.[365] Growth retardation caused by ergot feeding was more marked on a low-protein diet.[261] Crude casein and fatty acids in a carbohydrate-free, mineral-supplemented and vitamin-supplemented diet were essential to effective desoxycorticosterone treatment in adrenalectomy.[739] A low-carbohydrate diet reduced the effectiveness of desoxycorticosterone more than that of whole cortical extract.[246]

An acidosis-producing diet made rats more resistant to strychnine;[275, 898] however, this variation largely disappeared with intramuscular and intravenous administration.[12] Maintained on the same diet, rats of various strains showed uniform tolerance to red squill,[882] but not to thiourea.[203] Fluctuations in dietary calcium intake influenced the toxicity and storage of lead, and storage of arsenic was also affected by the calcium and phosphorus ratio in the food.[305] Foods, especially fats, hinder the absorption of certain drugs, such as alcohol; however, sometimes the reverse may hold, namely, that fats and oils in noncathartic doses, acting as solvents, may unduly favor the absorption of some other substances, such as phosphorus and rotenone.[16] The problem may even be more complicated than that, because in contrast to increased acute toxicity when olive oil was given with derris and rotenone, addition of fat (Crisco) in case of young rats prevented growth retardation and tissue damage.[15] Paraffin oil may take up certain vitamins and remove them from the alimentary tract. To avoid these complications and to prevent inclusion of inert material in the body weight, fasting is preferable in acute dosage determinations; hence food is often withheld, usually up to 24 hours preceding an experiment, and only water is supplied. However, even variations in the periods of fasting within this limit modified the insulin response,[358] and increasing the length of fasting increased the sensitivity to quinacrine.[736] Epinephrine hydrochloride gave irregular mortality responses when administered orally to nonfasted rats, whereas the results with the ascorbate were regular both in nonfasted and fasted rats;[504] fasting predisposed to carbon dioxide depression at low temperatures.[39] On the other hand, water deprivation up to 48 hours did not influence the toxicity of intraperitoneally administered nicotine.[330] A diminished food intake may be due to chronic poisoning, and the effects of the consequent semistarvation may inadvertently be ascribed directly to the poison, as was cautioned in the case of nicotine[905] and similarly in the case of lesser weight gains during sulfamerazine medication.[895] As to *flavoring,* most rats preferred alcoholized soup treated with scatol to ordinary soup flavored with orange, whereas normally ordinary soup was preferred to alcoholized soup by many rats.[833]

State of the Drug. The *purity* of a drug, the *form* in which it is administered and the *potential hydrogen ion concentration* (*p*H) of its solution all modify dosage. Thus a solution of crystalline sodium Amytal was found more potent than one of supposedly equal concentration made from Amytal, due to the difficulties of avoiding slight decomposition and to inability to convert all of the Amytal into the sodium salt.[817]

Of special importance is the fact that the toxicity of certain elements depends upon their valence; thus an equivalent amount of arsenic was more toxic in trivalent than in pentavalent form.[268] Even with the same valence, the nature of the anion may greatly modify the toxicity, partly due to difference in ionization (see Ferric and Ferrous Salts). Change into the sodium salt not only increased the toxicity of the medicine by increasing the alkalinity, but did so to a different degree with different compounds (sulfapyridine, sulfathiazole).[498]

Other modifications may alter the intensity and duration of action, as combining protamine with insulin.[465] Selenide was about half as toxic as the same selenium concentration from wheat and corn.[259] Slight lowering of the intended *p*H, which was 9.5, caused separation of traces of Amytal from a solution of sodium Amytal with loss of hypnotic power.[822, 915]

Stability varies greatly among freshly prepared solutions. Thus, epinephrine solutions quickly lose strength when exposed to air; acidity, cooling and exclusion of air increase the stability. Solutions of neoarsphenamine showed increased toxicity within a few minutes, hence should be protected from the air by using freshly boiled distilled water and a layer of mineral oil.[601] Sodium succinate solutions also should be freshly prepared;[647] likewise, fresh solutions of sodium fluoroborate should be made daily to avoid hydrolysis.[549]

In view of conflicting reports upon the stability of some aqueous barbiturate solutions,[398, 506, 625, 635, 822, 915] it seems preferable to prepare fresh solutions at frequent intervals and to exclude the carbon dioxide of the air; keeping sodium pentobarbital solution in a syringe was found helpful in preventing precipitation.[774] Although in some cases solutions may be stable over long periods of time, as in the case of di-isopropyl fluorophosphate,[455] in general it seems advisable to diminish deterioration of drugs by keeping them in sealed, dark containers in the cold and to exclude oxygen and other active gases by replacement of air with nitrogen. Suitable precautions should be taken to prevent absorption of moisture and decomposition during the weighing of such hygroscopic substances as hexobarbital soluble, lobeline sulfate or chloral hydrate, by weighing in a counterbalanced flask intended to hold the solution, or weighing the original sealed ampul and subtracting the tare weight; the latter procedure is specified in case of digitalis assay by the Pharmacopoeia of the United States. Danger of actual spilling of solution during injections of a series of animals can largely be avoided by placing the solution in the center well of a clean, discarded, broad-based paste jar, which a struggling or escaping animal cannot overturn.[394]

As to *concentration,* a 10% solution of sodium Amytal was more uniform in action and about 50% more effective as an anesthetic than a 2% solution, hypodermically administered;[625] however, the stronger, very alkaline preparation often causes eschars that heal poorly.[399] Likewise, in case of tetracaine, low concentrations were less toxic;[567] similarly, the fatal dose of choline hydrochloride was only half as great with a 2% compared with a 20% solution[388] and the anticonvulsant action of certain steroids was less marked with low concentrations.[794] With yellow oxide of mercury it required a 25-fold increase in concentration in the ointment to double the skin penetration, and reduction in particle size increased the penetration of mercury.[479] Concentration is also a factor in potassium cyanide toxicity.[419] However, it should be kept in mind that higher concentrations of various poisons in food or drinking water may make either so obnoxious that rats will scarcely eat or drink;[2, 393] also, that dilute solutions permit greater accuracy of measurement in case of potent substances.

The *solvent* must be considered with regard to the amount of drug it can dissolve, to drug stability, to absorption and to its own toxicity or other undesirable features. In general, alcohol solutions are more stable than aqueous preparations. Likewise Amytal deteriorated more slowly in ethylene glycol than in water.[635] However, alcohol itself is a drug and in some cases will increase the toxicity of the substance under study, partly by facilitating absorption from the stomach. Because the rat often shows very great resistance to poisons, the amount of alcoholic solvent may be near the fatal dose of alcohol itself, as in the case of tincture of digitalis. Not only must the factor of pain be considered when irritating solvents are used, but the relative toxicity of the solvents must also be considered. Thus propylene glycol is much less toxic than ethylene or diethylene glycol.[346, 398, 741] As to choice between distilled water and saline solution as a solvent, it has been found that rats died in greater numbers and more rapidly when a 1.8% sodium chloride solution was used as a solvent for ouabain in place of distilled water.[396] Calcium ions also are important in the action of cardiac glucosides.

Subcutaneous injections of sesame oil caused a decrease in the weight of the prostate and seminal vesicles in immature male mice and hence may obscure the effects of small amounts of a hormone dissolved in it.[169] However, though a solvent such as amylene hydrate was in itself hypnotic, it slowed the absorption and thus decreased the toxicity of rectally administered tribromoethanol, another depressant.[43] Likewise propylene glycol slowed the absorption of intramuscularly administered dihydroxypropyl bismuthate.[901] Solutions in oil are given to diminish irritation and to slow absorption of such substances as sex hormones; however, reports on the relative merits of oils as solvents are conflicting. Testosterone was so poorly absorbed from paraffin oil or glycerin as to be almost inactive;[581] administered percutaneously, it was more effective in alcohol than in oil, lanolin or ointment;[618] desoxycorticosterone also was more effective when given in alcohol-water suspension than in oil;[246] on the other hand, mineral oil, though unabsorbed, was a satisfactory vehicle for iodine.[216] Camphor disappeared in a day, while the olive oil, used as a solvent, remained at the site of injection for months;[80] similarly, injections of dimercaprol over a number of days were unsatisfactory owing to accumulation of peanut oil.[284] Also, cysts formed around intramuscularly-injected fixed oils and remained at least one year; abscesses frequently formed when almond oil was injected, rarely in case of cottonseed oil; the cyst wall was more durable with sesame oil than with corn, olive or peanut oil.[234] On the other hand, sesame and corn oil were found superior to peanut and cottonseed oil for intramuscular injection.[110] Failure to obtain high mortality with high doses of dichloro-diphenyl-trichloroethane (DDT), given orally, was attributed to the large volume of corn oil used as the solvent.[910] With sesame oil as the solvent for diethylstilbestrol, large fluctuations in response occurred.[560]

Dosages of Metrazol were somewhat higher when dibutyl succinate was used as the solvent instead of water.[314] Inclusion of certain fatty acids in the solvent, sesame oil, markedly increased the activity of testosterone.[581] Addition of acacia not only prevents undue irritation and diminishes the toxicity, but was also found to protect against circulatory disturbances in intravenous medication.[635] Skin penetration of mercury was greatest with oleic acid and propylene glycol; petrolatum, mineral oil and lanolin were poor vehicles.[479] Certain features of the use of oils as solvents have been discussed under diet.

Administration. In the case of certain barbiturates and of uranium tartrate the oral dose was very much greater than the parenteral.[254, 320, 911] This also holds for several snake venoms, and immunity was not produced by oral administration.[98] Although the oral dose was greater than the subcutaneous one with dihydro-beta-erythroidine, it was 40% smaller in case of beta-erythroidine.[858] With insoluble metallic salts, such as lead sulfate, the typical poisoning manifests itself very slowly with daily oral administrations, whereas following subcutaneous injections, it begins more promptly. In case of most hormones, these are inactive or much less active when given orally. However, several steroid hormones were equally well absorbed from the peritoneal cavity and the intestinal tract;[747] also, adrenal cortical extract was reported more effective when given in the drinking water than when injected once or even thrice daily.[246] In case of certain arsenicals, the oral method is far superior, therapeutically, to the intramuscular or intravenous one in rat trichomoniasis.[620] Thiourea has been reported to be effective by mouth, ineffective if given subcutaneously;[157] however, using larger doses, others have employed the subcutaneous route of administration,[539] and in olive oil suspension this poison has been found effective when given intraperitoneally.[203] An actual difference in effect according to the method of medication has been reported for iodine, which has a thyroxinlike action when given subcutaneously but only an iodide effect when orally administered.[217]

Similarly with emetine the hypodermic route may be preferable to reach the dysenteric amebae harbored in the intestinal wall. Usually the action is quicker and the fatal dose smaller with intravenous administration; but with neoarsphenamine the tolerated single dose was reported to be greater by vein than by subcutaneous injection.[790] At times slow absorption may be desired, as with sex hormones or insulin. Intraperitoneal administration corresponds to a slow intravenous injection, provided the drug is not unintentionally introduced into the stomach, intestines or even a full bladder. With repeated intraperitoneal doses of alcoholic solutions the alcohol was found injurious to the intestines.[204] Better survival from operations following oral administration of Amytal was found than with subcutaneous medication.[634] The effective dose of estrone and estradiol benzoate was much smaller by intravaginal than by subcutaneous administration.[795] Subcutaneous implantation of testosterone caused anestrus, but in the spleen it was ineffective.[83]

In case of a volatile substance, such as iodine or mercury, the vapor may be inhaled. Thus a small but detectable amount of mercury was inhaled from a site of cutaneous application.[480] To prevent the rat from licking a treated part of the skin, estradiol was applied to the shaven, inaccessible suprascapular area, and such animals were kept in individual cages, lest other rats remove the applied medicament.[178] It must also be remembered that a single large dose may be quite inactive, whereas the same dose may prove effective, or even lethal, when divided over several days, as reported in case of thiouracil,[274] sex hormones, thyroxin and parathyroid hormone.[219] Further, in regard to hormone administration, prolonged medication with large doses may produce less effect than that seen after smaller ones, partly explained by formation of so-called antihormones (see Androsterone).[838] Thus with thyrotrophin or thiouracil the expected effects on the thyroid gland were transitory.[272, 273] Also, with small doses of phenyl thiouracil over a period of 15 days the rats lost weight, whereas with larger doses the weight remained constant or increased slightly.[842]

The rate of absorption of estrogenic or androgenic substances from tablets is proportional to the surface area at any one moment; it occurred as rapidly, or more rapidly, with intraperitoneal as with subcutaneous administration; crowding several tablets into one subcutaneous pocket retarded absorption.[168, 638] Several sites of injection were more effective than one with digitoxin.[440]

The *rate of intravenous injection* may profoundly alter the effect. Thus the fatal dose of sodium amytal declined sharply with increased speed of injection,[822] and the safety margin varied widely in mice, depending on the rate of injection used. This necessitated a determination of the most favorable ratio.[686] A very rapid injection of a few cubic centimeters of an animal's own blood has been reported to produce widespread disturbances, named "speed shock," with a possible early death.[384]

Animals Used. A large *number of animals,* both in the therapeutic and fatal ranges, obviously insures more reliable dosage values; unfortunately, the exact number of rats and other pertinent information used in establishing a dose is not always given in the literature. Burn has discussed the number needed for a given dispersion in the animal response,[119] and Munch has presented a table and discussions dealing with this.[609] Methods for greater accuracy in the determination of dosage-mortality curves from a small number of animals have been proposed by Bliss,[86] by Dragstedt and Lang, and by Behrens (see [912]); numerous articles have more recently appeared dealing with designs of tests and application of the principles of statistical analysis, such as those by Gaddum[282] and by Pugsley;[655] very helpful has been the publication of tables and nomographs by which sufficient accuracy may be obtained without carrying out extensive calculations (see texts at end of Introduction). Unfortunately, it has become necessary to warn investigators that the use of even apparently simple methods of dosage determination requires an understanding of the principles involved.[908a] As to number of animals, in spite of considerable individual variations from day to day, the number of rats that convulsed following a certain dose of thujone was remarkably constant when forty rats were used.[704]

In regard to *species, strain* or *variety* of rat, inbred rats gave much greater uniformity with red squill than those of mixed stock.[909] Albino rats were many times less susceptible to diphtheria toxin than the Eastern cotton rat.[435] Marked differences in the choline requirements of two strains of hooded rats,[165] in the reaction to thiopental, and to N-methyl-pentobarbital in three strains of rats,[405] and in the amount of estradiol required to produce estrus in seven inbred strains of rats have been reported.[740] Denver rats were somewhat more sensitive to morphine than Stanford rats.[769] The widest variations noted were in resistance to thiourea in case of wild Alexandrine or Norway rats as compared with tame Norways from two different colonies; even between the latter there was an elevenfold difference.[203] Estrone produced significant increases in adrenal and pituitary weights in fully developed rats, but not in "runts."[766] Inbred untreated albino rats gave more uniform hair growth after depilation than those of mixed albino or colored rat origin and hence were better suited to studies on hair growth promotion by various endocrines.[235]

Although intralitter and interlitter rat responses to hexobarbital were practically alike,[390] significant interlitter differences have been reported in responses to gonadotrophins,[32] to quinacrine feeding,[260] and to selenium;[259] see also vitamin D under body weight. Consequently, symmetrical distribution of *litter-mates* among the several groups under study should be made together with appropriate analysis of variance.[32]

As to *sex,* the adult male rat has been reported distinctly more resistant to red squill,[909] nicotine,[399] picrotoxin,[396] ethyl-o-ethylphenyl-urea,[387] sulfanilamide,[467] arsenic trioxide,[4] hexaethyl tetraphosphate,[333] various steroids in regard to their depressant action,[236, 744, 793] dichloro-diphenyl-trichloroethane (DDT),[237] and to selenium.[259] Such greater sensitivity on the part of the female rat has also repeatedly been reported for certain short-acting barbiturates;[397, 399, 405, 586, 625] however, in the case of sodium pentobarbital this is still controversial,[136, 625] and sex-difference was reported absent with thiopental.[405] Whereas adult males developed tolerance to pentobarbital much more readily than females, the latter detoxified this drug on a par with the males once tolerance had been developed.[401] The degree of the greater female susceptibility to strychnine[650] has been reported to depend in part upon the method of administration[881] and has even been reported to be largely absent with intravenous or intramuscular administration.[12]

Caffeine stimulated all phases of maze performance during learning and relearning in female rats but not in males.[99] Females were considered less suitable in studies on analgetics, because males were tamed more easily and tended to lie quietly while heat was being applied to the tail.[769] Only female rats showed a moderate transient elevation of systolic blood pressure with prolonged stilbestrol medication.[381] Greater responsiveness of the females to thiouracil has been reported,[539] but this was not verified by other investigators.[274, 842] Finally, males gained weight more readily on discontinuation of prolonged salicylamide or acetyl salicylic acid administration.[422]

On the other hand, female rats were found more resistant to lead salts,[132, 602] cortin,[425] ouabain,[403] cadmium,[429] sodium arsenite,[269] cocaine,[210] and egg-white injections.[488] Males only showed bladder stones in chronic ethylene and diethylene glycol experiments.[603] Females were reported more resistant to choline deficiency than males.[165, 309] Ergot feeding caused more pronounced retardation of growth in the males.[261] Greater resistance of female rats to morphine also has been reported,[537] but the evidence for this claim has been considered inadequate.[250] Ferrous sulfate was better absorbed by females, iron and ammonium citrate by males.[354]

Normal *sex life* has been reported to increase resistance of both sexes to many substances in prolonged experiments.[4] Regarding sex life, tolerance developed much more often in shorter tests upon sodium pentobarbital only during the periods of *pregnancy* and lactation in mature female rats; various types of sex life and cohabitation had no effect upon male resistance to this hypnotic.[397, 401] The essential elimination rate of strychnine was about twice as great in male rats compared with females, which again eliminated one third more than pregnant females.[353] Pregnant rats tolerated doses of thyroid and thyroxin which in nonpregnant controls caused rapid loss of weight and death.[94] On the other hand, pregnant rats have been reported especially sensitive to combined administration of tyramine and phenylethylamine.[2] Alloxan administered by vein antepartum promptly reached the fetuses, but only the mothers became diabetic; the young were raised by normal mothers.[271] In general, it seems advisable to use only fully mature, unmated rats and to keep records of the sexes and ages in ordinary dosage determinations.

Concerning the *size* and *age* of the rats, their characteristics must be determined with reference to each drug.[152] In regard to the barbiturates, heavier but also older rats were found more sensitive to ortal[315] and to Seconal.[152] On the other hand, rats weighing between 160 and 180 Gm. needed 25% more barbital to produce the same duration of depression as shown by those between 100 and 160 Gm.[458] No such differences were seen with various drugs, especially sodium pentobarbital, in large-scale experiments upon length of depression and mortality in adult rats of either sex with considerable body-weight variations but of rather uniform age (animals from four to seven months old).[396] The same apparently held true for rats over nine months of age.[136] Likewise in rats that gained weight slowly as they advanced in age from four to nine months, no increased sensitivity could be seen with biweekly administration of hexobarbital.[390] The newborn rat was more susceptible to pentobarbital than the adult rat,[242] and very young rats have been reported more sensitive to pentobarbital,[309] to Amytal[54] and to hexobarbital (males only).[390] However, rats from one to two months old developed tolerance to pentobarbital much more readily than adult females and ultimately detoxified much more of this drug per unit of body weight than adults of either sex.[401] Maximum resistance to pentobarbital was reported for rats weighing from 50 to 200 Gm.[405] In case of thiopental the median lethal dose was slightly higher for rats weighing from 75 to 99 Gm. than for those over one year old, weighing from 220 to 566 Gm.[137a]

Changes in age may not affect various criteria of depression equally. Thus, while the dose required to abolish the righting reflex with vinbarbital was rather constant between the ages of one and nine months, the fatal dose declined as the rats became older, hence also the safety margin.[401] In the case of morphine, newborn rats were more resistant to acute respiratory failure than adults, but the fetuses were about as susceptible as their mothers; however, the newborn were much more susceptible to the late effects of morphine than were adults.[154] High sensitivity to morphine in very young rats has also been reported by others.[152, 288, 871]

The anticonvulsant action of selected steroids was greater in smaller rats.[794] Very young rats also were more susceptible to procaine,[224] aniline, colchicine, pyrocatechin, and hydroquinone;[871] sulfanilamide;[167] and strychnine.[650] With strychnine, minimum resistance occurred at emergence from the crawling stage.[725] However, with amphetamine the fatal dose of the young rat was six times that of the adult;[223] young rats were also more resistant to arsenic trioxide;[339] neoarsphenamine;[698] ergot;[474] nostal;[680] and to urethane and ethyl alcohol, but had similar resistance to other alcohols, chloral hydrate, paraldehyde and barbital.[871]

In the case of codeine, rats six weeks old gave about the adult response.[649] Rats up to two months of age withstood several hundred times the dose per unit of body weight of thiourea that killed adults of the same strain.[203] Suckling, wild Norway rats were seven times as resistant to alpha-naphthylthiourea (ANTU) as adults, and resistance decreased with increasing body weight.[202] Concerning this poison and also dichloro-diphenyl-trichloroethane (DDT), it should be kept in mind that young, rapidly growing rats, who consume more food in proportion to body weight than older, less active rats, will receive a greater daily intake per unit of body weight when these poisons are mixed with the food in fixed proportion.[257] Likewise older male rats were much more sensitive to ephedrine, propadrine, and amphetamine, but not to vonedrine, Tuamine, Privine, or phenylephrine;[885] larger rats were also more susceptible to quinacrine.[758] The average fatal dose of epinephrine was higher in heavier or older than in lighter or younger male rats.[900]

In case of alloxan, it has been reported that the effective dose was significantly lower in old rats,[111] but also that younger male rats tolerate it less well than older ones.[570] Tolerance was developed to small doses of nicotine by growing rats only; the males alone lost weight.[63, 64] Sometimes the age factor is more complicated; thus 10-day-old rats were most susceptible and 5-week-old animals least susceptible to phenol poisoning; adults took an intermediate position.[193] Similarly, female rats from 1 to 1½ months old died from smaller ephedrine doses than those 2 weeks or 2 months old.[152]

Concerning the optimum age and weight, this differs with the substance under study. The most uniform results with certain diuretics were obtained with young, unmated rats weighing from 100 to 120 Gm.,[427] and male rats weighing from 140 to 240 Gm. are recommended for determination of antidiuretic potency of posterior pituitary extract.[119] Prime of life and sexual maturity are important in assay of follicular hormone.[116] Optimal ages for percentage in weight change responses to suitable hormones were 14 days for the testes, from 14 to 18 for the prostate and 26 for all other organs studied; histologic changes were demonstrable earlier than the greatest weight changes.[654] The initial weight was a factor in vitamin D assay, hence initial weight differences should be minimized.[431]

Previous medication with a given drug may greatly alter the subsequent dose. With short intervals between administrations, increased *tolerance* frequently occurs, as with some barbiturates,[397, 586] and notably in the case of alcohol;[871] also with certain organic—but not inorganic—arsenicals.[461] With some organic arsenicals cross tolerance was also noted;[461] even delayed death following nostal administration may largely be prevented by previous small doses of this hypnotic,[397] and cross tolerance has been demonstrated with certain related barbiturates.[680] Sometimes an animal may show decreased tolerance, or "*education*," to actions of a drug, strychnine being an example; repeated cocaine administrations made rats increasingly sensitive to this drug;[210] hypersensitivity also followed administration of aniline, colchicine, pyrocatechin and hydroquinone.[871] Temporary hypersensitivity with longer intervals between medications has been noted with sodium pentobarbital.[586]

It is beyond the scope of this chapter to discuss the modifications incurred by dietary deficiencies, by removal of glands of internal secretions or of other organs, by excessive fatigue, by exposure to cold[743] or by disease. Likewise we must exclude the often very large changes in the effects of a given dose due to antagonism, synergism or modified action by previous medication with other drugs than the one under study.

Having considered all the factors discussed, an unusual response also possibly may be due to *error,* as in calculation of the drug concentration, in making the solution, in measuring the individual dose, or reading it from a prepared table, in recording the body weight (100 Gm. error in a long series!), or in injection technic (intraperitoneal injection instead of subcutaneous into a struggling rat; injection into a viscus). One should rule out beforehand any rat that does not appear to be in every way normal. Even with such preliminary examination obscure diseases may exist in some of the animals used. This cannot always be established afterward because many pharmaceutic agents cause pathologic changes. In conclusion, if at the end of an experiment no valid cause for a discrepancy can be found, any unusual response must be included in the results.

DOSAGES

Explanatory Notes. The drugs have been arranged alphabetically according to names commonly accepted in this country. However, to avoid repetitions, the selected androgens have been placed together. In the case of chemical names we have tried to place the more important component first, as arsenic, trioxide, or glycol, ethylene. For the sake of compactness we have omitted a considerable number of as yet unidentified active principles of various body tissues and glands. In the case of the anterior pituitary body, information may be secured from Burn[119] and the extensive reviews by Van Dyke[862] and Van Dyke *et al.*[864] (see also Chapter 12). An asterisk (*) indicates that the dose has been confirmed by Professors T. Sollmann of Western Reserve University and P. J. Hanzlik of Stanford University.*

Unless otherwise specified, doses are milligrams per kilogram of body weight or per rat, if the latter is stated; doses expressed as gammas, millimols, cubic centimeters, or units likewise are per kilogram of body weight or per rat, if such is specified. The methods of administration have been abbreviated as follows: *oral,* by mouth or stomach tube; *subc.,* subcutaneous; *im.,* intramuscular; *ip.,* intraperitoneal; *vein,* intravenous; and *inhal.,* inhalation. Doses ranging from minimum effective to maximum nonlethal have been included under the heading *therapeutic or typical action.* When a dose is shown without qualification in the table under the heading *fatal,* such a dose was designated originally as the fatal or the minimum lethal dose. A number after the lethal dose (LD) represents the percentage of rats killed by the given dose. Thus LD 50 would be the average or mean lethal dose and the LD 100 the surely fatal dose. When the duration of a test is indicated, as in the expression "48-hour test," it will be understood that a single dose was administered and that the effects were studied for 48 hours only.

Under *remarks,* condensed information concerning therapeutic and toxic actions is found in the same order as the doses; a period separates the effects of typical and of lethal doses when both are given. When only the fatal dose appears, one may usually expect "typical" effects with from 25 to 40% of this, or less in cases of curative actions, as with certain arsenicals.

Although only a limited number of substances could be included in this table, the reference or references for each substance usually list suitable doses for agents with similar actions, often meaning for less important new derivatives, not generally available. Doses for agents with opposite action frequently may also be found in these references. Furthermore, valuable cross references are readily secured.

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* We are grateful for their kind permission to use this information from their laboratory guide.[786] We also wish to thank many other friends for counsel and for much valuable material placed at our disposal.

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Acacia	8-16 cc., vein		6%; against shock	786
Acetanilid	50-500, oral; in 2% acacia; 16-hour fasting		Methemoglobinemia; acetophenetidin less effective	501
	200, oral; 6 times weekly		Tolerance; 10-week test	772
		400, same	Toxic to blood and growth	
		840, oral	Majority died	797
	400, ip.	800, ip.	Antipyresis. LD 50	770
Acetarsone (Stovarsol)	1000, oral; fine suspension		Degree of amebic dysentery reduced	432
	5 x 500, oral during 72 hours			
	150, oral	Over 4500, oral	Minimum effective in trichomoniasis; 24-hour test	620
	100, im.	140, im.		
	Over 100, vein	300, vein		
	120; 140, vein		Trypanocidal; tolerated	786
	100, vein	150; 300, vein	Tolerated. LD 50; 100	589
Acetate, Sodium	95-109, oral; females; daily in 2 doses, 8 hours apart	122-150, same	Maximum tolerated for 2 weeks. Most died in a week; weight loss, blistering of paws; red nose and whiskers	359
Acetic Acid	10-20 per rat, ip.		Adrenal hypertrophy; other acids, urea and creatinine also effective	73
		3310, oral	Observed 6 days; neutralized with NaOH before administration; 18-hour fasting	896
Monochloro-		76, oral		
Dichloro-		4480, oral		
Trichloro-		3320, oral		
Acetone	1.8 cc., daily (2.5% in drinking water) for 4 months		Anorexia; stunted growth	783
Acetonitrile (Methyl Cyanide)		0.5-3.9, subc.	Cyanide symptoms; depression	420
Acetophenetidin (Phenacetin)	750-1000, oral; in 5% acacia	2000, same	Tolerated; cynanosis, respiratory depression	107
	560, oral	2600, oral	Analgesia in 50%. LD 50	697
Acetyl-beta-Methylcholine			See Metacholine	

Drug	DOSES — Therapeutic or Typical Action	DOSES — Fatal	Remarks	Reference
Acetylcholine Chloride (See also Carbachol)		2500, oral 250, subc. 22, vein	Cholinergic stimulation; each was the LD 50	588
		250, ip., as acetylcholine; 1-hour test	LD 50; nitrate ester of choline perchlorate: 2.5 mg./Kg.	139
Acetylcholine Iodide	20 ip., or 0.5 after physostigmine 0.1-0.15, vein, or 0.002 after physostigmine		Chromodacryorrhea (bloody tears)	829
Acetylene	78%, inhal.	90%, inhal. for 2 hours	Narcosis in 15 minutes	682
Aconitine Crystals		0.10; 0.14; 0.25, subc.	MLD; LD 50; 100	610
		0 08, ip.	About LD 50	
	0.19, ip.	0 25, ip.	Heart and respiration greatly disturbed	174
Actinomycin A		1.0, subc., ip. or vein; 7-day test	Weakness, languor and diarrhea set in slowly; ataxia and convulsions	690
		0 050, ip ; daily for 6 days	Respiratory failure, functional and gross pathologic changes	
Adalin			See Carbromal	
Adenine Sulfate	25-100, oral; 140-240-Gm. males; fasted 18 hours	1249, oral; same; 3-day test	Potent diuretic; theobromine-type action; formoguanine and melonine also active. LD 25	510 512
Adiphenine (Trasentine)		17.3, vein; 200-250-Gm. rats	LD 50; 8 morpholine derivatives given	147
Adrenaline			See Epinephrine	
Adrenochrome, Iodo	10, im. or ip., per large male, daily for 3-4 days; in propylene glycol		Blood-pressure lowered in experimental hypertension only; no toxic effects	632
Alcohol, Allyl, Vapor		50, 200; 1000 parts per million of air; inhal.	Multiple hemorrhages; fatal in 30; 2; 0 12 days, respectively	529
Alcohol, Benzyl		1700, ip	Paralysis; acute or delayed death	252
Alcohol, Ethyl	2.5-5.0%, sole liquid source		Interference with growth	783
	10%, same		Anorexia; greater growth interference	
	10%, same for 163 days		Fully normal weight gains	623

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Grain Alcohol or Whiskey	8-16%, sole liquid source		Normal growth; less activity; no intoxication or habituation	676
	5% in tap water *ad lib.;* 8-week rats; for 304 days		Liquid intake reduced with considerable weight reduction (dehydration); noisy breathing; sluggish maze performance	100
		7400, oral; 18-hour fasting	Acute deaths; mostly young males	896
		12.96 cc., oral	LD 50	491
	7.5%, sole liquid source for 12 weeks		Normal growth	45
	15%, same		Growth retarded, but less so with whiskey	
		3500, 5000; 6000, ip.	About LD 10; 50; 60; delayed type of death, more so with straight run whiskeys	45
Alcohol, Isopropyl	1%; 5% in drinking water for 27 weeks; females	0.5%; 2.5%, same, males	Remained well; growth somewhat subnormal. Some found dead; growth normal	491
		6.73 cc, oral	LD 50	
	5% in tap water *ad lib.* for 304 days; 8-week rats		Liquid intake reduced; considerable weight reduction (dehydration); noisy breathing; sluggish maze performance	100
	2.5% in drinking water for 2 successive generations		Normal reproduction and embryonic development; early growth retardation of 1st generation offspring overcome by 13th week	495
Alcohol, Methyl		2.5% in drinking water*	Stunted growth; few deaths	783
		5%, same*	Anorexia; severe weight loss; fatal in a few weeks	
		12 cc., oral*	Coma; death in a week	789
Alizarine Red S (Sod. Sulphalizarate)	39-62, ip.; 2% sol.		Demarcated bone and teeth calcification	156
Alloxan	175-200, subc.; hooded rats	300-350, same	Rapid, selective necrosis of Islands of Langerhans and severe diabetes	214

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
Alloxan, *cont.*	175, subc.; 48-60-hour fasting		Fasted rats more sensitive; larger rats need longer fasting	439
		200, subc., males	90% died overnight; smaller, repeated dose more satisfactory	520
	200, ip.		Diabetes in most rats; hooded rats more resistant; subcutaneous medication unsatisfactory	296 481
	25, vein; 7-week females; alternate days for 4 weeks		Impaired glucose tolerance	753
	25, vein; 1- to 3½-week intervals; 6 doses		Progressive, marked decrease in glucose tolerance	
	40, vein		95% became diabetic	481
		40, vein; normal and pregnant females	Estrus intervals prolonged; fetuses died at 12th day and were resorbed in diabetic survivors	184
Alloxan Monohydrate	250; 400, ip.		Diabetes and azotėmia; transitory renal damage though diabetes persisted	66
	40, vein		Diabetes in most rats; no renal damage; most satisfactory method	482
Alpha-Naphthylthiourea (ANTU)	0.005-0.05% in food; domestic Norway rats; 14-day test	0.1-10% in food for 24 hours	Temporary tolerance and refusal. All died	677
	0.019-0.08% in food for 2 years; male weanlings		Stunted growth; spectacle eyes, thin hair and deformed legs and feet; pathologic changes in organs and bones	257
		5 ip.; in 5 or 25% propylene glycol	LD 50; marked hyperglycemia and glycogenolysis	213
		10, ip., in 25% glycol	LD 100	212
		58, ip.; suspended in olive oil	Wild Norways; LD 50's in: Sucklings	202
		43, ip.	Weanlings	
		22, ip.	Young	
		8.1, ip.	Young Adults	
		7.7, ip.	Adults	
			Marked pulmonary edema in adults	

Drug	DOSES Therapeutic or Typical Action	 Fatal	Remarks	Reference
Aluminum Chloride (See also Chapter 5)	600 mg./Kg. of food		Normal and healthy from weaning to maturity	844
		7000-8000, subc.	Fatal in 1-3 days; 2 rats used	850
Sulfate	0.4% in food		Protected against fluoride	549
Alurate, Sodium (Numal)	52.5, subc.	175, subc.	Min. effective in abolishing ear reflex	627
		100, ip., as the acid	LD 50	254
Alypin			See Amydricaine	
Amidon (Dolophine; Methadon; Adanon) Hydrochloride	0.01-0.1% in food for 28 days; 100-Gm. rats	0.2-0.5%, same	Growth slightly depressed. Distinct depression with 0.5%; non-specific pathologic changes	730
		95, oral	LD 50; analgesia	329
		100, subc.	Same	
	1, ip.		Analgesia for 1-2 hours	730
		9.2, vein	LD 50	329
	5, ip., daily; 27-day rats	24, ip. 37, ip.	Good growth; partial tolerance developed LD 50: normal; tolerant rats, respectively	932
AMINES			SEE ALSO DIAMINES	
Amine, Monobutyl, Normal		600-1500, subc *	Circulatory depression; antipyresis; increased peristalsis; convulsions	786
Amine, β-m-methyloxy-phenyl-n-propyl		40, vein, males	LD 50; active bronchodilator	303
Amine, Phenylethanol Sulfate		140, vein	Vasoconstriction; cyanosis and convulsions	823
Amine, Phenylethyl	20-40 per rat, subc.; daily for several days		Lowered liver glycogen, especially in the females	2
		300, subc.	Vasoconstriction	608
Amine, Tetrahydro-*beta*-naphthyl	5-7.5, subc.		Suitable fever-producing doses	144
Aminobenzoic Acid, Para (PABA; see also Sulfaguanidine and Thiouracil)	1% in purified ration		Normal growth and reproduction in females	240
	3% in food; males; 19-45-day tests		Thyroid hyperplasia; increased resistance to lowered barometric pressure	297

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
Aminobenzoic Acid, Para, *cont.*	1% in drinking water; weanling females; 10-day test		29 Gm. weight increase; potent thyroid inhibitor	28a
	3 per rat, oral; daily for 30 days; in 20% alcohol		Cured graying produced by deficiency diet in black rats	24
	1000-2000, oral or ip.; 100-Gm. rats; daily for 3-4 days		Reduced carbarsone fatalities, but not trypanocidal action	708
	750-1000, oral or ip.; daily for 1-5 days		Reduced renal damage by tryparsamide or neostibosan	350
		10,000, oral	About LD 90 in immature, LD 45 in mature rats	685
	400-500, ip.		Limited protection against several bismuth compounds	874
	15-1000, ip., within 1 hour before and 15 min. after arsenicals		Varying degrees of protection against several, but not all arsenicals	707 709 710
		7.5 per 30-day male, ip; daily for 48 days	Body-growth retarded; gas in intestines; 2/9 died	811
As Sodium Salt	132, vein; 80-120-Gm rats; fasted 16 hours		Protected against neoarsphenamine; best when in same solution	528
Para, Meta or Ortho	750, oral or ip.		Efficient antidotes in arsanilic acid or carbarsone poisoning	706
Aminophylline (Theophylline Ethylendiamine)	9; 34, oral; in food, 40-day test with females. 60 with males		No toxic effects	839
	100, oral	200, oral	Tolerated. Fatal within 24 hours	547
	20, oral with 50 cc. 0 9% NaCl per Kg		Marked diuresis	
	50-240, vein		Tolerated	
Aminopropiophenone, Para (PAPP)	10, subc.; females; in 0.1% propylene glycol		Evisceration increased the methemoglobinemia	832
		55, ip ; young males	LD 50; methemoglobinemia	859
Aminopyrine (Amidopyrine; Pyramidon)	300-1000, oral	1500, oral	Analgetic action qualitatively different from that of opiates	239
	750, oral	1700, oral; 2-day test	Less than 50% showed analgesia. LD 50	697
	150, subc.		Lowered temperature even in normal rats	409

Drug	DOSES: Therapeutic or Typical Action	DOSES: Fatal	Remarks	Reference
Ammonium Chloride	250, ip.		Acidosis; diuresis	459
Amphetamine (Benzedrine) Sulfate	2, oral		Increased activity; no tolerance	917
	3, subc.		4-hour stimulation; no tolerance	737
	20-150, subc.		Excitement; tolerance; increased liver glycogen	223
Hydrochloride		35, subc.	Majority of adults died	
		200, subc.	Majority of young rats died	
		165, subc.	LD 50's, 190-250-Gm. males;	887
		39, subc	300-350-Gm. males;	
		30, ip.	same	
Amphetamine *d*-methyl Chloride (Pervitin; Estimulex)	0.05-0.1, subc.		Faster, slightly irregular respiration	90
	0.5, subc.		Deeper, much faster respiration; increased activity for 21 hours	
	1.0, subc.	1.5-3.0, subc.	Increased motility. Convulsions	
		9.5-95, ip.	Fatal range; irregular results	376
	0.6, vein		Motor restlessness	679
A. *l*-methyl	128, subc.		Increased activity for 21 hours	628
Amydricaine (Alypin)		200, subc.	Local anesthesia	424
		10-15, vein; 2% sol.	Convulsions	406
Amylene Hydrate (Tertiary Amyl Alcohol)	930, rectal or subc.		Complete sedation	48
	1100, same	1400, same	Weak anesthesia. LD 85	
	500, 1000, ip.		Sleep; reflexes lost	847
		2000, 1350, ip.	Acute; delayed death	
Amylocaine			See Stovaine	
Amytal Sodium	225, oral	400, oral	Minimum anesthetic dose	820
	100, subc	150-200, subc.	Anesthesia, females	625
	200, subc.		Anesthesia, males	
		230, subc.	Majority died	818
		115, ip., as the acid	LD 50	254
ANDROGENS IN SESAME OIL				

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
Androstane-3 (α)-17 (trans)	100-500 gammas per rat, subc.; daily for 10 days		70-Gm. rats; injected 2-3 weeks after castration; seminal vesicles 43-120 mg.; controls 14	846
Androstane-17-one-3 (α)-ole (Androsterone)	200-1000, same		Vesicles 20-136 mg.	846
	5000, same		Same 14 mg.	580
Androstane-17-one-3 (β)-ole (Isoandrosterone)	1000, same		Same 14 mg.	581
Δ 5-Androstene-17-one-3 (β)-ole (Dehydroandrosterone)	100-500, same		Same 10-16 mg.	846
Δ 4-Androstene-3-one-17 (cis)-ole (Testosterone)	1000, same		Same 15 mg.	581
Δ 4- Androstene-3-one-17 (trans)-ole (Testosterone)	50-200, same		Same 42-124 mg.	581
	5000, same		Same 50 mg.	580
		50 per 100-Gm. female, ip.; 5 were given every 30 min., in peanut oil	Slow, progressive anesthesia in 50%; males less sensitive (see Progesterone)	744
T. Acetate	10-200 gammas per rat, subc.; daily for 10 days		Vesicles 30-383 mg.; controls 14	582
	0.5, subc.; females: daily for 15 days		Pernoston depression shortened	402
T. Propionate (Perandren; Neo-Hombreol; Oreton)	5-200 gammas per rat, subc ; daily for 10 days		Vesicles 20-444 mg.	630
	5000, same		Same 22 mg.	580
	0.5-20 mg. per 4-week female		Premature vaginal opening; estrus	703
	0.5 per day-old female, subc.; thrice 1st week, then 1-3 times weekly		Ovarian cysts and urolithiasis with 40-180 mg. total	750 285
	1 subc., daily for 10 days		More castrated males, normal and spayed females developed tolerance to pentobarbital	402

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
	0.5 per rat, subc.; daily for 10 days, or 2.5, same for 10-20 days		Changes in weight and chemical composition of corpus luteum essentially those occurring during pregnancy	476
	0.2-0.5 per 60-day male, subc.; daily for 25 days		Cellular changes in anterior pituitary gland	876
	40, total per adult female, subc.; 16-21-day tests		Diminished pituitary gonadotrophic activity	475
	20-40, total per male rat, subc.; 10-day test		Diminished estradiol damage to testes	428
	5 per rat, subc.; daily up to 7 days		More resistant to small mercuric chloride doses	516
	0.5 per 2-8-day female, subc.; thrice weekly; 1.5-5.5 total		Large, lactating mammary glands	557
	0.125-3.0 per 1-day male; thrice weekly for 4 weeks; in peanut oil		Severe reduction in libido and sexual potency; less effective if started later	906
	1, im.; thrice weekly for 7 weeks		Testes diminished; transitory seminal vesicle hypertrophy in normals, continuous in castrates	800
Testosterone, Methyl	5, subc.; 68-Gm. rats; daily for 76 days; in peanut oil		Fatty and other adrenal cortex changes	746
Δ 4-Androstenedione (3, 17)	25-500 gammas per rat, subc.; daily for 10 days		Seminal vesicles 14-126 mg.	846
	5000, same		Same 27 mg.	580
	70; 100, ip.; 100-150-Gm. females		Minimum anticonvulsant and hypnotic doses, respectively; males more resistant	794
Aniline Hydrochloride		460, ip.; 125-Gm. males	LD 50; methemoglobinemia	859
Antileprol			See Chaulmestrol	
Antimony Ethyl Tartrate	2 per 100-200-Gm. rat, subc.		Trypanosomes disappeared in 1-2 hours	840

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Antimony Potassium Tartrate (Tartar Emetic)	13, vein	50, vein	Minimum trypanocidal dose	868
	1.6, as radio-antimony, ip.		Increasing blood-concentration of Sb beginning at 8 and lasting at least 72 hours	621
Antimony, Other Compounds			See Stibamine, Stibanose and reference 355	
Antipyrine (Phenazone)	220, oral	1800, oral; 48-hour test	Analgesia in 50%. LD 50	697
Antithyroidin-Möebius	200 per 200-Gm. male, oral; daily for 5 days		Basal metabolism depressed	629
Apothesine Hydrochloride		20, vein	Procaine-type of action	406
Arsanilate, Sodium (Atoxyl; Arsamin)	12; 120, vein		Trypanocidal; maximum tolerated	713
Arsenate, Sodium		14-18, as As, ip	At least LD 75	268
Arsenic Trioxide (See also Chap. 5)	0.00005-0.0005, in food, oral; daily*		Checked appetite and retarded growth	784
	0.0015-0.005, same*		Body weight loss	
	27; 215 mg., as As, per Kg. of food		Fertility and fecundity normal	602
		8, subc.	About LD 50	340
Arsenite, Sodium	25 mg., as As, per Kg. of food for 100 days		Normal growth	269
	50, same		Slight growth retardation; males more sensitive	
	1.5, as As, subc.; daily		Detoxicated 1.0-1.5 mg./Kg. of Se.	604
		4.25-4 75, as As, ip.	At least LD 75	268
Arsenophenylglycin	20, vein		Minimum curative; given 4th day after *T. lewisi* inoculation	469
	90, vein; 150-180-Gm. males		Maximum tolerated by infested rats	
	150-200, vein; 125-150-Gm. males		Same, normal rats	
Arsenoxide			See Oxophenarsine	
Arsphenamine (Arsenobenzene; Salvarsan)	4.8 (1.5 as As), vein		Minimum trypanocidal dose	869
		95; 140; 178, vein	Minority; half; majority died	691 786

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
		154, vein	LD 50, crystalloid fraction	
		104, vein	Colloid fraction	
Di- or Monosodium Salt	175-200, subc.		Tolerated	786
	100, ip.*		Trypanocidal	
	113-125, ip.		Tolerated	
	10-30, vein		Trypanocidal	
		100-160, vein*	Toxic and lethal	
Ascorbic Acid (Cevitamic Acid; Vitamin C) (See also Chap. 6)	10% in basal diet; 21-day males		Diarrhea and poor growth	35
	50-100, vein; hooded rats; fasted 24 hours		Increased sensitivity to alloxan	502
As Sodium Ascorbate	360-560, vein; 100-Gm. rats; fasted 16 hours		Marked protection against neoarsphenamine when given simultaneously	528
Aspirin			See Salicylic Acid, Acetyl	
Atabrine			See Quinacrine	
Atophan			See Cinchophen	
Atoxyl			See Arsanilate	
Atropine Sulfate	700, subc.	750, subc.	Tolerated; cholinergic paralysis	904
	500, ip.	600, ip.	Tolerated	
	8-10 per 150-170-Gm. male, vein		Protected against lethal damage induced by mechanical tumbling	914
Avertin			See Tribromoethanol	
Bacitracin		190 (6700 units), in 1 cc. water, ip.; males	LD 50; all died in 24 hours; only slight kidney involvement	733
BAL			See Dimercaprol	
Barbital Sodium (Medinal; Sod. Veronal)	190, oral or ip.	400, oral	Anesthesia	320
	200, subc.	400, subc.	Minimum anesthetic dose	849
		350, ip.		320
		300, as the acid, ip.	LD 50	254
	145-180, ip.		Side position	499
		300-440, ip.	Reflexes lost	

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
Barium Carbonate		630-750; in food	Almost surely fatal	723
		20% in bait	Rapidly fatal	
Barium Chloride		335, oral	Majority died	723
	35, subc.	45; 178, subc.	Tolerated. LD 43; 89; brown rats	
Benadryl			See Diphenylhydramine	
Benzedrine			See Amphetamine	
Benzene (Benzol)	1 cc., subc.; daily		Anemia, leukopenia, emaciation and weakness	56
		1.25-1.75 cc., ip.	Convulsions; peritoneal and lung congestion	
	460-2440 parts per million of air; inhal.		Toxic symptoms	
Benzestrol (Octofolin)	0.8-1.0 gamma per spayed female, subc.		Positive estrus response; not toxic with prolonged overdosage	85
Benzoic Acid	1% in food + equal sod. bicarbonate for 20 days		Anestrus; ceased quickly when medication stopped	656
	750, ip.		Antidoted arsanilic acid and carbarsone	706
		1714, vein	Tremors and convulsions	334
Benzyl Benzoate		2800, oral	LD 50; piloerection, hind leg paralysis, spastic convulsions and respiratory failure	304
	10, ip. or subc.; 10% suspension		Antipyresis; slowed maze performance	536
Berberine Hydrochloride	20-25, subc. or ip.		Life prolonged in *T. equiperdum* infestation	738
Beryllium Nitrate (See also Chap. 6)		2000-5000, subc.	Thirst, depression and "stiff legs"	801
Biotin (Vitamin H; Coenzyme R)	1-2 gammas per weanling male, oral; 6 times weekly for 5 weeks		Biotin-deficient diet; incomplete cure of lateral graying	231
	1 gamma per rat, oral; daily; basal diet		Prevented spasticity and histopathologic changes	749
B. Methyl	1 gamma per 40-50-Gm. black rat, oral; daily for 5 months		1-2% sulfaguanidine in synthetic diet; better growth, more survivors and no chromotricia	554

Drug	DOSES: Therapeutic or Typical Action	DOSES: Fatal	Remarks	Reference
Bismarsen (Sulfarsphenamine Bismuth)	400, im.		Tolerated	459
		350-500, im.	About LD 3-16	660
Bismuthate, Sodium	90, subc.;* in suspension	200, subc.;* in solution 175, im.;* in solution 20, vein*	Tolerated	489
In Tri-isopropanolamine, Propylene Glycol and Water (Sobisminol Mass.)	300-650, oral;* daily (0.2-0.5% in drinking water) for 25 weeks		Young rats; delayed growth; practically noninjurious to viscera	343
	120, oral	240-720, oral	420* was about LD 50	344
	50, im.	100-250, im.	Minimum to surely fatal dose in all 3 cases	
	10, vein	11.5-25, vein		
Bismuth Thioglycollate Sodium (Thio-Bismol)	8-10, as Bi, ip.	10-12, as Bi, ip.	Tolerated	322
Boric Acid	0.5% in food for 10 days		Anestrus; uncertain with 0.4%	656
	Same for 37 days		Normal cycles when medication ceased	
	400, average, subc.; weanling males: daily for 30 days		Normal growth and blood-picture	276
	1000, subc.; daily		Sustained, incomplete estrus	656
Brilliant Green (Malachite Green)	5 per rat, subc.; 0.5% solution		Temporary *T. nagana* suppression; local necrosis	897
		5-8, ip.	Marked lung congestion; too toxic in leprosy	19
Bromide, Sodium (See also Chap. 5)	120, oral; daily; 3d-20th day of gestation		Maze learning slower in 61-day offspring	349
		3500, oral	LD 50; in 2% acacia	771
		1750, oral; daily	Listlessness; weight loss; average life 7 days	773
		875, same	Average life 20 days	
Bromural	400, oral: in suspension		Majority lay in side position	313
Bulbocapnine Hydrochloride	75, subc.		General depression; slowed heart rate	589
Butacaine Sulfate (Butyn Sulfate)		80, ip.	Convulsions of procaine type	459
		7.5-10, vein		406

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Cadmium Chloride	50 mg./Kg. of food or water		Reduction in hemoglobin and incisor pigment, but not in caries	290
	62.5 mg./Kg. of food		No visible effects	429
		125-135 mg., as Cd, per Kg. of food	Growth soon ceased; males died sooner; marked anemia	429 255
		250-1000, same	Anorexia; no growth; death occurred soon	
	62 mg., as Cd, per Kg. of food		Severe anemia; cardiac hypertrophy and other tissue changes	907
	10-25, as Cd, subc.		Usually tolerated	11
	1.0-1.5, as Cd, vein	3.0, as Cd, vein	Usually tolerated	
Caffeine	0.020-0.10% in food for 28 days; 100-Gm. rats		Little or no depression of growth	731
	0.3-0.5%, same		Growth depressed	
		100, oral; daily	Growth retarded; some died in 13 weeks	773
		200, oral; in 2% acacia	About LD 50	771
		233, oral, fasted rats	LD 50	731
	10-20, subc.		Markedly increased activity; theophylline superior	729
	80, subc.; daily		Weight stationary; temporary estrus disturbance in 2 weeks	893
	40-60, parenteral		Creatine much increased	61
		250, subc.	About LD 50's	545
		210-280, ip.		449
	50-60, subc. or ip.		Though excited, showed maze-test depression	534
		105, vein; fasted rats	LD 50	731
Caffeine and Sodium Benzoate	100, as caffeine, subc.	130, as caffeine, subc.	Feeble convulsions	93
Calciferol (Crystalline Vitamin D_2; see also Ergosterol, Cholesterol and Chaps. 5 and 6)	0.025; 0.028 gamma, oral		Antirachitic unit per vitamin-D depleted rat	124 308
	75,000 units per Kg., oral; adult rats; daily		Average survival time about six weeks	605

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Calcium Chlorate, Anhydrous		4500, oral	Average lethal dose	848
		625, ip.; M/2 solution	Same; convulsions and respiratory failure	
Calcium Chloride, Anhydrous (See also Chaps. 5 and 6)	22.2 Gm./Kg. of food		Tolerated; no intestinal disturbance	265
		4000, oral	Average lethal dose	848
		500, ip.; M/2 solution	Same; convulsions and respiratory failure	
	700, subc.; 21-day females	1000, subc.	Maximum safe; convulsions and respiratory depression	541
Calcium Gluconate		400, as Ca, oral; 6 days weekly; suspension	20% died in 70 days; heart, kidney and liver normal	744
Calcium Lactate	20, vein*		Counteracted sodium iodide inflammation	787
Calcium Malonate	440 mg., as Ca, per rat; in food; daily for 10-12 days		Weight loss; more Ca in muscle, heart, liver and, especially, brain	681
Camphor, Natural		2200, subc ; 20% in olive oil	Convulsions	158
	400; 600, ip ; 20% in olive oil		Mild; marked convulsions	589
	To 500, im ; in olive oil		No antipyretic action	101
Synthetic		1700, subc , 20% in olive oil	Convulsions	158
Cantharidin, Pure Crystals	40 gammas per spayed rat, subc.; 2-3 doses in a day		Estrogenic action	803
Carbachol (Doryl, Lentine; see also Acetylcholine)		40, oral 4, subc. 0.1, vein	Cholinergic stimulant, stable in body fluids; all are LD 50's	588
Carbarsone	200-250, oral; 24-hour test		Minimum curative dose in trichomoniasis	620
	1000, oral; fine suspension		Degree of amebic dysentery reduced	432
	5 x 500, oral, during 72 hours		Same	
	1000, oral or vein		Trichomoniasis cured	281
		6000-6500, oral	LD 33 in 10 days	280
	200, subc.; daily for 5 days		Minimum curative dose in trichomoniasis	281

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Carbarsone, *cont.*	200, im.		Same; in 48 hours	620
		400, im.	LD 33 in 30 days	
Carbarsone Oxide		510, oral 55, ip. 17, vein	LD 50's; liver, lung and kidney damage	22
Carbon Dioxide	5%, inhal.; at 5° C		Hibernation-anesthesia condition; fasting predisposed	39
	10%, inhal. (oxygen 21%) for 7 days; room temperature		Prolonged pentobarbital sleep	40
	11%, inhal. for 30 days		Lowered plasma *p*H; marked reticulocytosis	
	15%, inhal.		Maximum tolerated	
	23%, inhal.		Same if several days allowed for adjustment	
Carbon Monoxide	0.04% in air for 8 hours daily; 5 consecutive days a week		Normal growth; smaller, lighter litters; mothers failed to nurse young	809
		0.12% in air; 33° C.	Fatal borderline; rats very sensitive	91
Carbon Tetrachloride	0.5 cc., ip.; alternate days		Initial weight loss; liver injury; decreased plasma cholinesterase activity; prolonged hexobarbital depression	106
		70-140 mg./liter of air for 90 minutes; inhal.	Acute deaths; delayed deaths with even less	52
Carbromal (Adalin)	350, oral; in suspension		Majority lay in side position	313
Cardiazol			See Metrazol	
Caronamide (4′-carboxyphenylmethanesulfonanilide)	250, oral, in 0.54 cc.; no other liquid; 11 doses weekly for 3 months		Females; no hematologic or histologic damage	72
Carotene, β; International Standard (See also Chaps. 5 and 6)	0.6 gamma, oral; daily for 3 weeks		International unit; vitamin-A depleted rats grew	123
	2 gammas per 7-week rat, oral; daily		Fair growth and decreased mortality in such rats	251

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
	0.44 mg. + 0.2 mg. α-tocopherol per rat, im.; in 0.1 cc. Tween 80		More effective than oral administration in vitamin-A depletion	843
	Same, in sesame oil		Oral medication better	
	7 gammas per rat, oral; weekly for 5 weeks; in oil		Equipotent in insuring weight gain and curing A-deficiency opthalmia;	483
	75 gammas, same; colloidal		Vitamin A efficiency independent of administration method	
	15-20 gammas per Kg., oral; daily		Prevented vaginal cornification in A-deficiency	299
	80 gammas, same		Detectable storage	
Cascara, Fluid Extract	1/1000 perfusion in situ		Stimulated intestinal peristalsis	302
Cellulose Acetate Phthalate	5; 20; 30% in diet for one year; females		Reduced growth with 30% from mechanical interference with food absorption; tissues normal	390
Cellulose, Ethyl	1.2% in fox chow for 8 months; young rats		Apparently harmless	195
C. Methyl	1% in drinking water. same		Same	195
Cerium Chloride	2000. subc.		Tolerated; hybrids	348
		4000, subc.	Slow death; sloughing	
	- - .	50-60, as Ce, vein		562
Cetyl Pyridinium (Ceepryn) Chloride		200, oral 250, subc. 6 ip. 30, vein	Approximate LD 50's; local injury and inflammation; peripheral paralysis and central nervous stimulation	619
Cevine (Sabadinine)		77, ip.; 100-150-Gm. rats	LD 50; profuse salivation; tonic-clonic convulsions; prostration	569
Chaulmestrol (Antileprol)	30 cc., subc.	35 cc., subc.	Tolerated. Irregular respiration, depression; irritation	228
Chaulmoograte, Ethyl	30-35 cc., subc.	35-50 cc., subc	Tolerated. Pre-death depression and pulmonary congestion	228
Chaulmoograte, Sodium	2000, vein	3000, vein	Tolerated. Rats showed convulsions with chaulmoogra preparations	20

Drug	DOSES Therapeutic or Typical Action	 Fatal	Remarks	Reference
Chelidonine Sulfate		300-400, subc.*	Lower doses narcotic; relaxed smooth muscle	342
Chenopodium Oil		700, subc. 120, vein	Depression and convulsions; depressed peristalsis	742
Chiniofon (Yatren)	700-3000, oral; 24-hour test		Trichomoniacidal; tolerated	620
	125-1000, oral; fine suspension		Reduced degree of amebic infestation	432
	5 x 500, oral, during 72 hours		Same	
		600, subc.	Kidney damage; diarrhea	719
Chinosol			See Oxyquinoline	
Chloral Alcoholate	400, oral; semiweekly for 6 weeks	880, oral; fasted 16 hours	No pathologic changes. LD 50	4
Chloral Hydrate	400, oral; same	800, oral; same	No pathologic changes. LD 50	4
	500, oral	1100, oral	50% slept. LD 50	494
	200; 300, ip.	500, ip.	Minimum hypnotic, anesthetic and fatal doses	
Chloralose	55, ip.; males		50% in stage IV side position	218
Chlorobutanol (Chlorbutol; Chloretone)	400, oral	1000, oral	LD 0. LD 50	589
	100; 400, subc.	500, subc.	Sleep; narcosis. LD 50	
	110, vein	135, vein	LD 0. LD 50; fatal if drug caused rat to lie on side	499
Chloroform (See also Chap. 2)	0.1 cc./liter of air, inhal for 35 min.		Sublethal	161
		0.2 cc, same	Lethal in 11.2 minutes	
		5 cc. or higher, same	Lethal in 1.5 minutes	
	1 volume %, inhal.	Slightly more than 1 vol. %	Narcosis	363
Chlorophenol, Penta	About 3.9 per rat; in food; daily for 28 days		Retarded growth due to reduced food intake	192
		27, oral; in 0.5% stanolex fuel oil 78, oral; 1% in olive oil	LD 50's; motor weakness, asphyxial convulsions; cardiac and muscular collapse; hyperpyrexia and hyperglycemia	
Pentachlorophenate Sodium		211, oral; in water	Same	192
		80, subc., same	Same	
Cholanate, 3, 12-Dihydroxy-keto, Sodium	250-380, vein; anesthetized rats		Marked cholagogue action	392
		1800, vein	LD 50	

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Cholanthrene, Methyl	10 per rat; pellets in 20% paraffin put into sex organs or adnexae		Gonads highly resistant to carcinogenic action; castrate male adnexae sensitive	142
Cholate, Dehydro, Sodium	250-380, vein; anesthetized rats		Marked cholagogue action	392
Cholesterol, 7-dehydro, Irradiated (Vitamin D_3; see also Calciferol, Ergosterol and Chaps. 5 and 6)	0.0215 gamma, oral		Unit per rachitic rat	308
Choline	41.8 per 150-Gm. rat, oral; daily		Absolute protection against dietary hepatic damage	306
C. Chloride		3400, oral; 50-67% sol. 6100, oral; 20-40% sol.	Excitement, then depression; chromodacryorrhea; occasionally delayed death; various viscera engorged	622
		290-340, ip.; 20% sol.	LD 50; wide scatter in results	391
		370-380, ip.; 10% sol. 410-490, ip.; 4% sol. 590-750, ip.; 2% sol.	LD 50's	
Choline, Brominated		190, oral or 60, subc. for 10 days	Approximate LD 50's; stable in blood for 4 hours	172
Iodinated		700, oral or 200, subc.; same	Same	172
Cinchonine Hydrochloride	35-150, ip.		Most potent antipyretic of 7 cinchona alkaloids	143
Cinchophen (Atophan)	10, oral; in food; daily		Maximum uric acid riddance from liver	279
	200, same		Severe weight loss	
Sodium Salt of Cinchophen	150, oral; daily for 21 days		Marked liver necrosis with weight loss	36
	400, same for 7 days		Same	
		600, oral; daily for 7 weeks	Some died; weight loss; liver and kidney necrosis	
	400, oral; daily		No liver damage even with higher doses	37

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Sodium Salt of Cinchophen, *cont.*	600, same		Normal growth	
	800, same		Tolerated many weeks	
		1000, oral; daily	Died in a few days or weeks	
		500, im.	LD 33	659
Citrinin		67, subc. or ip.; 14-day test	LD 50; hyperemia of ears and feet; dyspnea, prostration, nasal discharge and decreased appetite	14
Cobalt and Sodium Citrate (Heldt's Salt; see also Chap. 5)		20, as Co, subc.	Minimum lethal dose	92
		30-40, same	Convulsions; fatal in 15-60 minutes	
Cobaltous Chloride	2.5; 5; 10, as $CoCl_2$, subc.; 200-Gm. rats; 6 times weekly for 8 months		Persistent polycythemia and increased erythrocyte thickness; no apparent toxicity	798
• Cocaine Hydrochloride		100, subc.	Convulsions; MLD	424
		250, subc.	LD 50; MLD	692
				717
	50. ip.	75; 150, ip.	LD 0. LD 50; 100	589
		80; 100, ip.	LD 50; 99	209
	10.5, vein		Convulsant hindleg position	224
		12.5; 17.5, vein	MLD; LD 50	406
				692
	1; 5, intracardially		No convulsions; most showed tonic-clonic convulsions	899
		7-13, intracardially	Fatal range	
Codeine (Administered as Salts)	1060, oral		Good analgesia	239
	42, subc., or 49, ip.		Average analgetic dose	
	63, subc.		Tranquilizing dose	42
		336. subc.	Majority died	49
	40, ip.		Righting reflex depressed	220
		106; 98, ip.	LD 50: males, females	649
	30 ip. or 10, vein		Increased reaction time of tail to heat by 5 seconds	183
	10-35, vein		Analgetic range	
C. Phosphate	175, subc.		"Standard" analgesia	239

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Colchicine	1, subc ; in 6.4% alcohol		Arrested cell division	263
Congo Red	140, vein	190, vein*	Curare antidote	675
Convallaria		32,000, subc.*	Cardiac poisoning	786
Copper Sulfate (See also Chap. 5)	5.1, as Cu, per rat; in food; daily for 4 weeks		Decreased growth and food-intake	102
		11.8, same	Increased Cu content in liver and spleen	
Cobefrine (Corbasil; Iso-adrenaline)		12, ip. or vein	Pulmonary edema; animals quiet	679
Coramine			See Nikethamide	
Coriamyrtin	0.3, subc.	1 0, subc.	Minimum convulsant dose	544 819
	0.2, vein	0.7, vein	Same	
Corticosterone (See also Chap. 12)	0.5-1.0 per adrenalectomized rat, subc.		Positive Eversede de Fremery test (normal muscle reaction restored)	667
Corticosterone, Desoxy	0 8, same		Same	667
	10, same; young rats; in sesame oil		Lived 9 days; controls only 6.5	576
Corticosterone, Desoxy Acetate	10, same		10-day survival	576
	20, same		12-day survival	
In Sesame Oil	10, per normal rat, subc.; daily for 20 days		Hypochloremia and adrenal atrophy; smaller doses caused only the former	745
	2-4 per rat, subc.; daily		Increased appetite for NaCl with consequent polydipsia	671
	20, subc.; daily		Protected against KCl poisoning; physiological atrophy of adrenal cortex	519
	1 per young hooded rat, subc.; daily for 7-41 days		Marked K loss in brain, muscle and liver, none in heart; slight Na increase in brain and muscle; water content of these tissues unchanged	916
	3 per baby female, subc.; twice daily for 42 days; 1% NaCl sol. to drink		Half the rats showed experimental arthritis	641

Drug	DOSES: Therapeutic or Typical Action	DOSES: Fatal	Remarks	Reference
In Sesame Oil, *cont.*	5 per normal 100-Gm. female, ip.; in peanut oil		Anesthesia in 50%; males less sensitive; see also Progesterone	744
Cresol, Meta		0.35 cc., oral	LD 50; convulsions	470
		2020, oral; in olive oil	LD 50's	193
Ortho		1350, same		
Para		1800, same		
Disodium Mono-orthocresyl Phosphate		800-1000, vein	Tonic and clonic convulsions	778
Monosodium Diorthocresyl Phosphate		800, vein	Same symptoms	778
Crystal Violet			See Methylrosaniline	
Cubé Root (See also Rotenone)	300 per Kg. of food for 150 days		Young males grew normally	331
		100 oral	Rats especially sensitive	835
		170, oral; aqueous	LD 50	331
		245, oral; oil suspension	LD 50	
Curare (See also Intocostrin)		1 per 50-Gm. rat, subc.	Death in 4-5 minutes	97
	3.0, vein*		Every 10 minutes until complete paralysis	786
Cyanamide		200-400, ip.	Cholinergic paradoxical mydriasis and porphyrin lacrimation; coma; death in 3-6 hours	50
Cyanate, Sodium	20-30, im.; 120-150-Gm. rats		Short drowsiness	81
	50-150. im.		Marked drowsiness and diuresis; livelier next day	
		310, im.; 100-150-Gm. females	LD 50; terminal convulsions	
		60, im.; daily for 2 weeks	2/24 died; continuous drowsiness; loss in body weight	
Cyanide, Potassium (See also Hydrocyanic Acid)		10-15, subc. 2.5, vein	Convulsions; fatalities varied with concentration	419
Cyclobarbital			See Phanodorn	

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
Cyclopal (5-Cyclopentenyl-5-allylbarbiturate) Sodium	75, oral; males; fasted 20 hours	205, same	Anesthesia in 50%. LD 50	860
Cyclopropane, Monochloro-	5-7; 2 6-3 vol. %; inhal.		Start of anesthesia; maintenance of same	360
Dichloro-	2.5; 1.4, same		Same two uses	
Cyclural			See Hexobarbital	
Cysteine Hydrochloride, Neutralized	1000, as cysteine, ip.; 10% in 0.9% NaCl sol.		Antidoted LD 100 of *alpha*-naphthylthiourea when given within 5 hours after latter	213
	304; 608 or 912, vein; *p*H 7.4		Prevented alloxan diabetes when given 2 min. prior, but not 3 min. after alloxan	481
l-Cystine	0.3-0.7% in food		Moderate protection against dichloroethane; *l*-cysteine also effective	369
Dagenan			See Sulfapyridine	
DDT			See Dichloro-diphenyl-trichloroethane	
Delvinal			See Vinbarbital	
Derris Elliptica (See also Rotenone)		100, oral; water suspension 75, oral; in olive oil	Respiratory paralysis, no gross pathology	16
Dextrose (*d*-Glucose)	500 per male rat, oral; once or twice at ½-hour interval; 50% sol.		Prompt lymphocytopenia, lasting 2-3 hours	227
		16,000, ip ; 40% solution	Severe shock, often convulsions; respiratory failure in 3 hours; graded degrees of shock with lower doses	668
Dial (Curral) Sodium	60, subc.	150, subc.	Ear reflex abolished	627
	60, subc.	110, subc.	Minimum effective dose. LD 50	870
Dial in Urethane and Urea (Ampuls)	10 subc	75, subc.	Minimum effective dose. LD 67	630
Diamine, Diethylene			See Piperazine	
Diamine, Metaphenylene		30-50 per young rat, subc.	Facial and pleural edema; no hydropsy with ortho- or paraphenylene diamine	373

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
D. M. Hydrochloride		80, subc.*	Hydrothorax	786
Diamine, Paraphenylene Hydrochloride		170, subc.*	Edema of face and neck	786
Diamorphine			See Morphine, Diacetyl	
Diasone (Disodium Formaldehyde Sulfoxylate Diaminodiphenylsulfone)	3000-7000, oral		Tolerated	662
Dibenamine (Dibenzamine; Dibenyl-*beta*-chloroethylamine) Hydrochloride	10, ip.		Marked protection of heart against epinephrine	658
Dibucaine (Nupercaine; Percaine) Hydrochloride		60; 80; 90, subc.	Local anesthesia	630 295 287
	0.2-1.0, vein	4, vein	Signs of paralysis; convulsions with above 0.5	441 630
Dibutoline (Dimethylethyl-*beta*-hydroxyethylammonium Di-n-butyl-carbamate) Sulfate	500, oral		Atropine-like actions; no toxic symptoms	249
		22, ip.; in 0.9% NaCl sol.	LD 50; labored respiration with 15; tremors and convulsions	
Dichlorodiethylsulfide ("Mustard Gas")		0.2, subc., as hydrochloride	Approximate LD 50 in several days; intestines filled with liquid	104
	0.1; 0.2; 0.4; vein; 50-Gm. rats; in propylene glycol	2, same	Reduced mitosis in regenerating liver; P^{32} uptake by liver cells normal. Fatal in 3-5 days	552
2, 4-Dichlorophenoxyacetic Acid (2, 4-D)	150-200, subc.		Myotonia lasting 8-24 hours	112
Dichloro-diphenyltrichloroethane (DDT)	0.025% in food for up to 24 months; weanlings; dry or in 10% corn oil	0.05-0.10%, same	Liver damage; some thyroid degeneration; necrosis in hindleg muscles	258 910

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
		0.08-0.12% in food for 6 months; young males	Depressed growth; increased liver and kidney weights; low liver oxygen consumption; stored in all tissues	477
	0.2% in food for 1-10 days		Increased liver slice oxygen consumption; ineffective in corn oil	426
	50, oral; daily; in 2 cc. corn oil		Anorexia; lesser weight gains; liver enlargement	187
		150-200, oral; in olive oil	Approximate LD 50	777
		200-300, oral; in peanut oil; freshly emulsified	Most died in 5-24 hours; tremors at 24 hours in survivors	643
		140-300, oral; in corn oil	Irregular absorption; anorexia and severe weight loss; tremors and clonic convulsions	910
		2 cc. of 1% special emulsion per 250-Gm. rat, ip.	Fatal in 12-72 hours; chromodacryorrhea, salivation, tremors; muscle and nerve changes	134
		50, vein; in peanut oil; fresh emulsion	LD 94; tremors spread caudalward; most died in 3½ hours	643
		150, inunction; daily for 90 days; 30% in dimethylphthalate	Anorexia, severe weight loss; tremors, clonic convulsions; secondary infections and moderate leukocytosis	211
Dichloroethane			See Ethylene Dichloride	
Dichloropropane			See Propylene Dichloride	
Dicumerin (Dicumerol)	8 per rat, oral; 1-2 doses; in 1% tragacanth emulsion		Diminished prothrombin activity in 24-48 hours	523
β-Diethyl-aminoethyl-amino-ethyldiphenyl-thioacetate	50-100, subc.		Spasmolytic action	663
		2720, oral 1500, ip.	Approximate LD 50's; clonic convulsions	
Diethyl-amino-methyl-benzodioxane	5 per rat, ip.; 0.5% solution		Reduced blood pressure in renal "hypertensives," not in normals	711
Diethylstilbestrol (Stilbestrol; see also Benzestrol)	0.00095 per sprayed 75-Gm. rat, subc.; in olive oil		Estrus in 50%; three times estrone strength	457

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Diethylstilbestrol, *cont.*	0.00062, same; ¼ dose given each 12 hours; aqueous		Estrus in 50%; had 1.5 times estrone strength	
	0.25 per rat, subc.; thrice weekly; in sesame oil		Increased spontaneous activity; decreased weight in old males	411a
	10 per 30-day rat, subc. implantation		Inhibited growth of body, gonads, prostate and seminal vesicles; dipropionate more effective in males	568
	0.002-0.1 per young male, subc.; daily for 1 week		Increased tolerance to lowered barometric pressure	185
	0.1 per rat, subc.; daily; in sesame oil		Changed phosphatase activity within 28 days; increased metabolism after 6 weeks	113
	0.05-4 gammas per 25-day female, subc.; daily for 4-7 days; in sesame oil		Multiple stimulation effects in hypophysis	34
	1 per rat, im.; daily for 24 days; in cottonseed oil		Moderate, transient systolic blood pressure rise in females only	381
	1 per female, oral; daily for 44 days; in sesame oil		Liver degeneration	323
D. Dipropionate	1, same, for 54 days		Same effect	323
Digitalis Purpurea		3300; 3600, vein	30-50; 100-200 minute infusion; urethane anesthesia; other cardiac glucosides given	375
Digitoxin		100, subc.; in 70% alcohol	1 site of injection	440
		18, same	5 sites	
		2.5-3, vein; in alcohol, methylpiperidon and water	1 min. injection; fatal within 2 days	
		11.6, vein; in 40% alcohol	20 min. average injection time	
		15.5, same	71 min. average	
Dihydranol			See Heptylresorcinol	
Dihydromorphinone (Dilaudid) Hydrochloride	17.6, oral		Analgesia in majority	239
	0.88, subc.		Average analgetic dose	
	0.9; 2.0-2.2, subc.		Respiratory depression; complete tranquilization	796

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
	2, subc.; ascending to 20, daily for 5 weeks		Addiction and growth retardation	
	1.7, ip.		Average analgetic dose	239
	0.88-1.76, vein		Brief analgesia; irregular responses	
Dihydrotachysterol (Hytakerol)		1.0; 1.12; 1.25, oral; 7½-9-month rats; daily	Median survival times: 34; 24; 15 days; more toxic than calciferol	527
Diiodo-oxyquinoline (Diodoquin)	1000, oral; fine suspension		Degree of amebic infestation reduced	432
	5 x 500, oral during 72 hours		Same	
Dilantin			See Diphenylhydantoin	
Dimercaprol (Dimercaptopropanol; British Anti-Lewisite; BAL)	12.5, im., 1 hour after gold salt; in peanut oil and benzyl benzoate		Prolonged survival time with fatal gold sodium thiosulfate doses; 2 doses within 5 hours more effective	505
	60-80, im.; same solvent	100-120, 140, im	Safe. Some die; most die; liver and kidney damage predispose to BAL	133
	20-60, subc (in peanut oil); or ip. (in water); single or repeated doses		Antidoted mercuric chloride, sod. arsenite and tartar emetic; no effect on stibanose, but increased mortality with some other Sb compounds	284
		10, im.; in ethylene glycol	LD 50	262
p-Dinitrobenzene		56, ip.; 125-Gm. males	LD 50; methemoglobinemia	859
Dinitro-Orthocresol	5-20, subc.		Pyrexia	827
		10; 12.5; 20, subc.	MLD; LD 50; 100	
D-O, Sodium	0.00625% in food for 105 days; 54-Gm. males		Normal growth and food intake	13
		0.0125%, same	60% died; growth and food intake increased in survivors	
		0.05%, same	LD 100	
		30, oral	MLD	
	10-15, subc.	20, subc.	No toxic effects. MLD	
		25, subc	Hyperactivity, depression and pyrexia	
Dinitrophenol, 1-2-4	0.001-0.04% in food		Normal growth	825
	0.06-0.12%, same		Weight loss	
		0.08-0.24% in food	Increasingly shorter life; no pathology	
	5-15, subc.		Pyrexia	827

Drug	DOSES Therapeutic or Typical Action	 Fatal	Remarks	Reference
Dinitrophenol, 1-2-4, *cont.*		15; 25.5; 50, subc.	MLD; LD 50; 100	
	15, ascending to 40, subc.; twice daily for 3 weeks		Pyrexia; decreased activity; normal growth	338
Diodoquin			See Diiodo-oxyquinoline	
Diphenhydramine (Benadryl) Hydrochloride	72-158, oral; average daily from food intake for 28 days; 100-Gm. males	266, same	Normal weight gains. LD 50	175
		500; 545, oral 474, subc. 42, vein	LD 50's; complex excitation	321
Diphenylhydantoin (Dilantin; Epanutin; Phenytoin) Sodium	1000, oral		Mild incoordination and spasticity	319
	1500, oral		Prostration, rigor; sensation to pain persisted	
	2200, oral		Maximum tolerated dose	
	135, ip.		Increased electrical threshold of convulsions	245
	100, as the acid, ip.; 95-Gm. males; daily for 30 days		Normal growth; organs normal histologically	453
	250, ip.	500, ip.	Tolerated. 3/6 died	
		280, ip.	Spasticity, convulsions; some slow deaths	317
		160, vein	MLD	319
Divinyl Ether			See Vinethene	
Dolophine			See Amidon	
Doryl			See Carbachol	
Dyes			Various dyes given; see also individual dyes	833
Emetine Hydrochloride	2.5-20, oral 5 x 0.62, oral during 72 hours		Degree of amebic infestation reduced in recently weaned rats	432
		12, subc.	Smallest surely fatal dose	878
		7.6; 10.0; 13.4, vein; fasted 16 hours; 1% sol.	LD 1; 50; 100; young adults	791
Eosin		500, ip.	More sensitive in sunlight	229

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Epanutin			See Diphenylhydantoin	
Ephedrine Hydrochloride	60, subc.; 300-500 Gm males		Persistent rectal temperature rise	889
		300-320, same 650, subc.; 190-250-Gm. males 165, ip.; 300-500-Gm. rats	LD 50's	887
		181; 83; 83; 166; 112; 70; 69; 112, vein, females	LD 50's for ages: 14 days; 1; 1½; 2; 4; 6; 12; 24 months; convulsions; immediate death	152
E. Sulfate	100, subc.; daily for 2 months		Tolerated; various viscera normal	208
Epinephrine (Adrenaline) Hydrochloride	0.05 per rat, subc.; daily for 2 months		Tolerated; various viscera normal	208
	0.2, subc.; fasting rats		Oxygen consumption increased	140
	0.0005, vein		Uterine inhibition; phenobarbital anesthesia	451
		6.83; 8.96; 9.71, subc.; males	LD 50's in 50-100-; 100-150- and over 150-Gm rats	900
		6.36, subc. 3.07, im. 0.24, vein	LD 50's; 120-180-Gm. rats, both sexes; absorption and toxicity increased by sod. bisulfite addition	672
		2.5-7.5, subc.; 1-2 deposits	13-83% mortality; stored in heart in modified form	657
		2 ip. or vein	Pulmonary edema; rat quiet	679
Levorotatory		10-20, subc.	Most died; pulmonary edema	176
Dextrorotatory		80-100, subc.	Relatively less toxic	
Epinephrine, N-Methyl (Methadren)		105, subc. 50, ip. 5-6, vein	LD 50's	807
Equilin	0.5-0.7 gamma per spayed rat, subc.; in sesame oil		Estrogenic rat unit; given over 2 days	630
Erbium Nitrate		30-35, as Er, vein	Hemoglobin not affected	562
Ergonovine (Ergobasin; Ergometrine; Ergostetrine; Ergotocin)		500, subc	Hypothermia; oxytocic action	700

Drug	DOSES Therapeutic or Typical Action	DOSES Fatal	Remarks	Reference
Ergosterol, Irradiated (Ergosterine; Synthetic Oleo-vitamin D; Viosterol; see also Calciferol and Cholesterol)	1 mg. per rachitic rat, oral		International standard unit	124
	0.05 per normal rat, oral; daily		Normal growth and Ca-P relation; no toxicity	141
	0.0001 per rat, oral; daily		Curative in rachitis	466
	0.5, same		Various tissues calcified	
	1 0, same		Slight weight loss	
		5.0, same for 15 days	Marked weight loss; fatal in 15 days	
	11-17, same		Urinary calculi; survived 45 days	206
Ergot, Powdered, Crude	1-2% in adequate diet for 16 weeks; 3-week rats		Growth of males, but not of females, retarded	261
	5%, same		Ear tumors, especially on low protein diet; renal damage and corpus luteum hyperplasia	
*Ergotamine		100-150, subc.	MLD; gangrene in tail	700
E. Tartrate	17, subc.; 3-day test*		Fresh solution; consistent tail gangrene	515
		25-30, subc.	Diminished blood volume; hypertension; tail gangrene	311
Ergotamine, Dihydro Methanosulfonate	5; 10; 20, vein; daily for 16 to 40 days; 0.1% sol.		No tail gangrene; tolerance after 3 injections; main organs essentially normal	631
Erythrina Americana (See also Piscidia)		600-750, ip.* 100, vein*	Curare substitute	490
β-Erythroidine Hydrochloride		510, oral	LD 50; curare-like action	858
		1260, subc.	Same	
		100-200, ip.	Fatal range	
	5.0, vein		Paralytic dose	492
		39.3, vein	Salivation; bradycardia	146
E. Dihydro Hydrochloride		320, oral 230, subc.	LD 50's	858
		30-37.5, ip.	Fatal range	

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
	2.0, vein		Paralytic dose	492
		8 9, vein	Salivation; bradycardia; curare-like action	146
Estradiol (Dihydrotheelin; Progynon) In Sesame Oil	0.4 gamma per spayed rat, subc.		Estrogenic unit	578
	5; 50 gammas, same		Estrus lasting 2; 4 days	
	50 gammas per 60-Gm. female; in 2 doses		Uterus 100 mg. on 4th day; control about 50	
	5 gammas per nursing mother, subc. twice daily for 16 postpartem days; in cottonseed oil		Marked inhibition of lactation; ineffective in spayed mothers	55
Estradiol Benzoate (Estradiol Monobenzoate; Progynon B) In Sesame Oil	0.05-0.1 per spayed rat, subc.		Cornification in at least 50%	795
	0.0005-0.006, same, but intravaginal		Same results; in 2 doses, 24 hours apart	
	0.75 gamma, same, subc.		Rat unit; given over 2 days	579
	5; 50 gammas, same		Estrus lasting 8; 15 days	
	50 gammas per 60-Gm. rat, subc.; in 2 doses		Uterus 130 mg. on 10th day; control about 50	
	125 internat. units per immature female, subc.; daily for 1-6 weeks		Loss in body weight; decreased water and organic contents of femur	509
	30 gammas per male rat, subc.; 6 days a week for four weeks; in olive oil		Increased body-fat storage under specified dietary conditions	514
Estradiol Dipropionate (Progynon D P; Di-Ovocylin; Dimenformon D) In Sesame Oil	0.75 gamma per spayed rat, subc.		Estrogenic rat unit; given over 2 days	578
	5; 50 gammas, same		Estrus lasting 12; 42 days	
	50 gammas per 60-Gm. rat, subc.; in 2 doses		Uterus 180 mg. on 10-20th day; control 50-60	
	1, as estradiol, subc.; males; daily for 10 days		Severe weight loss; testicular atrophy; lengthened hexobarbital depression; females more resistant	402
Estradiol, Ethinyl (Estinyl; Lynoral)	0.1-20 gammas per rat on shaven suprascapular skin; daily for 20 days; in absolute alcohol		Typical effects on pituitary, sex organs and adnexae in normals and castrates; estrus; some growth retardation	599

Drug	DOSES: Therapeutic or Typical Action	DOSES: Fatal	Remarks	Reference
Estriol (Theelol; Trihydroxyestrin)	91-100 gammas per primed, spayed rat, subc.; in 3 doses during 8 hours		Positive smears in 50%; given in corn oil	575
	0.25 gamma per immature rat, subc.; aqueous		Twice daily for 3 days; vagina opened in 60%	
Estrone (Theelin; Folliculin) In Sesame Oil	0.5-1.0 gamma per spayed young rat, subc.		Cornification in at least 50%	795
	0.01-0.02, same, but intravaginal		Same effects; given in 2 doses, 24 hours apart	
	0.7 gamma per spayed rat, subc.		Estrogenic rat unit; given during 2 days	577
	5; 50 gammas, same		Estrus lasting 2; 5 days	
	50 gammas per 60-Gm. rat, subc.; in 2 doses		Uterus 85 mg. on 4th day; control about 45	
	10 rat units per rat, subc.; daily		First 10 days after nidation prevented or terminated pregnancy	181
	50-200 gammas per rat, im.; daily		Some mammary gland cancers after total of 35 mg.; 2-3 mg. pellets more effective; acted in males and castrates	286
	1 gamma per immature rat, subc.; aqueous		Twice daily for 3 days; vagina opened in 60%	575
	0.1-0.25, subc.; 100-120-Gm. rats; twice daily for 5 days; in oil		Increase in pituitary weights similar to that in castrates; contrasts with findings of 2d reference	762 764
	Other technics		See 65, 117, 122 and 182	
Estrone Benzoate	5-10 gammas per spayed rat, subc.; in sesame oil		Positive smears in 50%	336
Ether (Ethyl Ether) (See also Chap. 2)	3.2 vol. %, inhal.		Anesthesia	363
Ethodin			See Rivanol	
Ethyl Bromide	3-7 vol. %, inhal.	7-10, same	Narcotic range. Fatal range	364
		2, same for 1 hour	Delayed death	
Ethylene	80-85 vol. % + O_2, inhal.	95 vol. % + air, inhal.	Anesthesia	682

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Ethylene Dichloride	6.2 mg./L. of air, inhal for 4 hours	Same, 7 hours	0/13 died. 4/20 died 0/15 died. 15/16; 20/20 died	368
(Dichloroethane; Dutch Liquid)	12.4, same; 1½ hours	12.4 same; 3½; 7 hours 6.2, same; 7 hours daily	Pulmonary congestion; some died in 2, all in 5 days; rough fur, unsteady gait, anorexia; later depression	
	380, subc.; in olive oil	500-1250, same	Safe. Fatal range	
Ethylhydrocupreine (Optochin; Numoquin) Hydrochloride	500-550, subc. 150, ip. 30-40, vein		Tolerated; pneumococcocidal	461
Ethylstibamine (Neostibosan)	50 per rat, im.		Complex leucocyte changes in trypanosomiasis	423
Eucaine (*Beta*-eucaine) Hydrochloride		15-25, vein	Cocaine type convulsions; cardiac and respiratory depression	406
Eunarcon		115, ip., females	Narcosis; males more resistant	399
Evipal			See Hexobarbital	
Ferric Chloride (See also Chap. 5)	93-186, oral		Corrosion	799
Ferric Citrate	1.12% in food; young males		Protected against weight loss and blood changes in lead poisoning	366
Ferric and Sodium Citrate		186-372, oral Same, rectal	Death after 2 hours Death after 5 hours	799
Ferrous Chloride or Sulfate		279-558, oral 140-279, oral	Died in a few minutes to hours Sulfate: died after 4 hours; chloride: after 48	799
Ferrous Gluconate	1, as Fe, per rat, oral; daily		Young rats with milk diet anemia; good hemoglobin response	669
	Same, im		50% better than oral	
Fluoride, Sodium (See also Chaps. 5 and 6)	0.1% in food		Stunted growth; reproduction normal, but young grew poorly; dental fluorosis; hair growth retarded	720 127
		0.2% in food; 23-day rats	Failed to grow	127
	15-150, in food; daily*		Chronic intoxication	786
	18, ip.; daily		Loss in body weight	645

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Fluoroacetate, Sodium (Compound 1080)		2.5, oral	LD 58	883
		3.0-3.5, ip.	LD 60	
		7.5, ip.; albinos	LD 100; death due to effects on heart or central nervous system	
		4.0; 8.0, oral; Norways	LD 73; 100	
		2.0, oral; Alexandrines	LD 100	
Fluoroacetate, Methyl		4.0, oral 5.0, subc. or im.	Approximate LD 50's; myotonic convulsions; prolonged depression and bradycardia	153
Fluoroborate, Sodium	0.1%, as Fl, in food		Nontoxic	549
	11parts per million, as Fl, in drinking water; fresh daily		Teeth striated in 5 weeks; stock solution underwent hydrolysis	
	7 p.p.m., same		No striations	
Fluorophosphate, Diisopropyl (DFP) In Peanut Oil		1.82, im.; 200-300-Gm. rats	LD 50; cholinergic symptoms; majority died within 2 hours	408
		1, im.	LD 6; marked reduction in blood and tissue cholinesterase	455
		0.1; 0.3; 0.5, im.; twice weekly for 24 weeks	5/36 died; toxic effects in survivors ascribed to prolonged cholinesterase inhibition	456
		4200 mg./cubic meter of air; inhal. for 1 min.	50% died in 48 hours	446
		150, same; 30 min.	50% died in 48 hours	
3-Fluorotyrosine	0.0005-0.0025 % in food		Inhibition of growth	103
		0.004% in food	Fatal in 2-22 days	
		12.5, subc.	LD 50; convulsions; extreme lethargy; death within 24 hours	
Folic Acid (Pteroylglutamic Acid; Vitamin M or Bc; Folvite)	3 per 40-50-Gm. black rat, oral; daily for 1 month; 1-2% sulfaguanidine in synthetic diet		Growth rate restored to normal; chromotrichial action	554
	25 gammas per rat; oral; every 4 days		Corrected blood dyscrasia in dietary granulocytopenia and improved the clinical picture	463

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
Formaldehyde Sodium Sulfoxalate (F.-hydrosulfite; Rongolite; Formopone; Hydrolit)	1000, oral* or 500, vein*		Immediate antidote in mercurial poisonings	695
	1000, ip.; daily for 4 weeks		No symptoms or visceral damage	
Fumaric Acid	0.1 or 1.0% in food for 2 years		Normal growth and viscera	504
Sod. Fumarate	8080, oral; divided dose during 3 days		Tolerated	
		8080, oral; single dose	3/5 died; fiery red lungs; weight loss in survivors	
	1620, ip.; hooded rats	2420, ip	Shock; changed fluid distribution	
Furacin			See Nitrofurazone	
Furan		3.04 vapor %	Salivation; fatty degeneration of liver	361
Gelsemicine Hydrochloride		0.10-0.12, ip. or subc.	Tetanic convulsions	412 786
		0.08, vein	LD 50	151
Gelsemium		2200, subc.	Weak coniine action	786
Gentian Violet			See Methylrosaniline	
Glucose			See Dextrose	
Glutathione	614, vein; neutralized to pH 7.4		Protected against alloxan diabetes when given 2 minutes beforehand	481
Glycerin (Glycerol)	20% in food for 11 weeks; 4-month rats		Normal weight gains	393
	10,100, subc. 5000, im.	15,100, subc. 7600, im.	Undiluted; depression; local tissue necrosis. LD 60's	108
Glyceryl Trinitrate (Nitroglycerin)	20, oral		Lowered blood pressure	225
Glycol, Diethylene	1.71; 3 42% in food for 2 years		Bladder stones in males only; slight chronic damage	603
		1; 3-10% in drinking water	Most lived 100 days; rapidly fatal	328 445
	0.5-2%, same		Impaired growth	393
		4%, same	Some died	
		15 cc., oral or subc. 7 cc., im. 5 cc., vein	Majority died; local irritation	328

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Glycol, Ethylene	1% in food for 2 years		Bladder stones in males only; slight chronic liver changes; testicular changes	603
		2% in food for 2 years	Some deaths during first year	
		1% in drinking water for 11 weeks	Impaired growth; about half died	606
	700, daily; drinking*		Impaired growth	346
	2200, same		Oxaluria; renal calculi	
		4400, im.*	Local irritation; depression	
		1.0-3.5 cc., ip.	Fatal range	633
		2200, vein*	General motor depression	346
Glycol, Polyethylene				
Mol. Wt. 200		28,900, oral	LD 50's; 90-120-Gm. males; congested intestinal tract; 14-day tests	780
300		31,700, oral		
400		32,800 oral		
	4% in drinking water of each, respectively, for 90 days		Congested intestinal tract; no harmful effects	
		16% in drinking water	M. W. 400 least toxic; few survived 90 days; liver and kidney pathology	
Glycol, Propylene	2.45-4.9% in food for 2 years		Slight chronic liver damage	603
		22,850, oral; 50% solution	LD 50	684
	1-10% in drinking water for 20 weeks		Growth and viscera normal	741
		25% in drinking water	Anorexia; quick death	
	30% in basal diet		Retarded growth	902
		40% in basal diet	Rapidly fatal	
	30% in stock diet		Mothers unsuccessful in weaning 2d generation young	
	18,900, subc.		Severe transient irritation; profound depression	670
		23,100, subc.	LD 60	
	11,000, im.	14,000, im.	Semicoma. About LD 50	741
	Saturated atmosphere for 1 year; 7-week males		Grew better than controls; no harmful effects	684
Glycol, Triethylene	Saturated atmosphere for 18 months; 7-week males		Better than control growth after 7 months; no harmful effects; normal offspring	684

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
		15,000-18,000, oral; 50% solution	LD 50	
Gold Chloride, Yellow	15, as Au, ip.; 9 doses in 2 weeks	23-50, as Au, ip.; one dose	Decreased plasma ascorbic acid; increased tolerance and diminished growth rate	919
Gold and Sodium Thiomalate (Sod. Aurothiomalate; Myochrysine)	25, as Au, subc.; daily for 3 days, starting with infection	200, as Au, subc.	Complete protection against streptococcus infection. Death in 4-5 days; severe renal injury	699
Gold and Sodium Thiosulfate (Sanocrysin)	1, as Au, per adult rat, subc.; daily for 14 days		Excreted mainly by kidney; protracted retention in liver, spleen, kidney and blood plasma	88 87
		30, subc.	Died in 2-3 days	505
		75, subc.	Usually died within 6 hours	
	3 per rat, im.	5 per rat, im.	Increased nonprotein N in blood	199
		20-35, im., alternate days	Resistant renal tubular cells in survivors, which now tolerate 100, im.	167
Gossypol "Acetate"	0.675% in food		Growth rate retarded	726
		20-50, ip.; in peanut oil	Anorexia, serous exudation	727
		35-50, as sodium salt, vein	Pulmonary edema	
Gramicidin	1000, oral		Nontoxic	688
	10, ip.	20-60, ip.	Safe. Fatal range; restlessness, depression and hypothermia	
	1.25, vein	2.50-5.00, vein	Safe. Fatal range; usually died within 24 hours	
Gramine (3-Dimethylamino-methylindole) Hydrochloride		63, vein	LD 50; tonic convulsions; exopthalmia and cyanosis	652
Guanidine Hydrochloride		500, subc.	Carbohydrate depletion; few died	585
		750, subc.	LD 100	
		150-300, as guanidine, ip.; 100-Gm. rats	Dyspnea and salivation	450
		100-200, ip.; 150-Gm. rats	MLD; see also Synthalin	
		250, vein		10

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Halibut Liver Oil	200 per young rat, oral; daily for 100 days		Rapid growth; no pathology; 10,000 Vitamin A, 400 D units per cc.	237
Heptylresorcinol (Dihydranol)	1%; crystals in food		Removed intestinal protozoa in 3 days	248
		1000; 1500, oral	About LD 20; 70	472
Heroin			See Morphine, Diacetyl	
Hexanol, 2-Ethyl		3.9 cc., oral	LD 50; marked anesthetic properties	389
		0.8 cc., vein	LD 50	
Hexeton (Homocamphin; Cyclosal)	35, ip.	45, ip.	Violent convulsions; camphorlike action	458
Hexobarbital (Cyclural; Evipal; Evipan; Hexobarbitone) Sodium	100, ip.	280, ip.	Anesthesia. About LD 50	546
		160-200, ip.	Males more resistant	442
				399
Hexylresorcinol (Caprokol)		750; 1500, oral	About LD 55; 75; antiseptic and anthelmintic	472
Hydantoin			See Diphenylhydantoin	
Hydrocyanic Acid (3/7 of KCN)		118 mg. per cubic meter of air; inhal.	Fatal in 12 hours; convulsions	786
Hydroquinone		300-350, oral	Methemoglobinemia; convulsions	76
Hyoscine			See Scopolamine	
Hypericin (from St. John's Wort)	20 per adult rat; subc.	40, same	Sensory stimulation; darkness protected	407
Inositol (Hexahydroxycyclohexane)	0.3% of ration of naturally occurring foods		Cured alopecia and promoted growth	173
	1% in purified ration; females		Normal growth and reproduction	240
	5 per rat, oral, 6 days weekly for 9 weeks		Prevented alopecia from synthetic diet + 1% succinylsulfathiazole; growth also improved	626
Insulin (See also Thiamine)	200 units, subc.; daily for 5 days		Tolerated by rats allowed food; ordinary room temperature	533
	20 units, same; fasting rats	20 units, same; fasting rats	Safe unless rats had lost 25% in weight by 1st day of insulin	533

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
	1; 4 units, subc.; fasted 24 hours		Shock in 2; 40%; 26° C.	212
	1/32-1/2 unit, subc.; fasted 24 hours		Best hypoglycemic range; 26° C.	398
	2.2 units, ip.; fasted 12-16 hours		Average convulsant dose; room temperature	
	10 units, subc.; adult males fasted 4 days; twice daily for 10 days		Gain in weight; greater food intake and fat deposition; less tissue water content	238
	20.7 units, vein; 100-Gm. rats; fasted 16 hours		Median convulsive dose; some died over night; 27-29° C.	150
Insulin, Protamine-Zinc		10-60 units per rat, subc.	Rats allowed food; prolonged hypoglycemia; anorexia	7
Intocostrin (Crystalline *d*-Tubocurarine Chloride)	0.50-0.75 units, subc. or im; 150-200-Gm. rats		Flaccid, but hyperactive to external stimuli	160
	1.0 unit, same		Muscle twitchings	
	1.25 units, same		Epileptiform convulsions	
Inulin (Alantin; Dahlin)	5% in food for 1 year; females		Grew somewhat better than controls	390
	20%; 30% in food		Tolerated; diarrhea and weight loss	
Iodide, Potassium (See also Chap. 5)	0.57% in food for 10 days		Protected against alpha-naphthylthiourea and thiourea	130
	0.252%, as I from KI or Lugol's sol in drinking water for 1-2 days; special stock diet		200-Gm. males; increase in LD 50 of alpha-naphthylthiourea, depending on amount of I taken in; protected against thiourea	129
	0.5-10 per young adult, vein	30-50, same	Metabolism depressed. Convulsions	194
Iodide, Sodium	0.1; 1.0 mg./100 Gm. of food for 2 weeks; immature rats		Protected thyroid against thiouracil and para-aminobenzoic acid	538
	50 mg., same		Similar action against thiouracil; synergistic sulfaguanidine hyperplasia	
	25, vein*		Pleural effusion	787
Iodine, Elemental	1.0 per young female, subc.; daily for 10 days; in mineral oil		Prevented thiouracil effects on thyroid	216
Iodoacetic Acid		50-70, subc.	Hypersensitivity; convulsions	337

Drug	DOSES Therapeutic or Typical Action	DOSES Fatal	Remarks	Reference
Iodobismuthite Sodium (Iodobismitol; Bismuth Sodium Iodide)	33-50% of MLD	135, im. (30 as Bi)*; 6% + 12% NaI in ethylene glycol 90, vein (20 as Bi)*	Tolerated. Depression; death within 6 days	347
Ipral			See Probarbital	
Isonipecaine			See Meperidine	
Isopral (Trichloroisopropyl Alcohol)	300, oral	1000, oral	Sleep in 50%. LD 50	125
Isoriboflavin	2 per weanling, oral; daily for 28 days		Inhibited growth-promoting action of suboptimal riboflavin intake	233
Kallikrein (Padutin)	2-4 units, intracardially; 35-55 seconds before cocaine		Cocaine toxicity diminished	899
	0.05-0.5 cc., vein*		Lowered blood pressure considerably	496
*Lactic Acid	0.1 cc. of 0.05-0.3%, im., exposed zone of innervation		Rapid destruction of most motor end plates	135
Laurylsulfate (Drene; Dupanol; Gardinol)	0.2% in drinking water	1%, same	Decreased growth rate. Fatal in 10 days	828
		150; 400, ip.	LD 50; 100	786
Lead Acetate	64; 512 mg., as lead, per Kg. of food		64: fertility normal; 512: males grew less; kidneys heavier	602
		130, ip.	Visceral damage	114
Lead Arsenate	3.53 mg., as lead, per Kg. of food		Anemia, anorexia, kidney damage; males grew less	132
Lead Carbonate	0.0007-0.015; in food; daily for 8 weeks*		Checked growth and appetite; a younger group tolerated more	785
		0.007-0.15; in food; daily	Some died in 9-17 weeks (probably from lowered resistance)	
Lead Nitrate	250, subc., as Pb; daily for 3-5 days		Diminished ascorbic acid in adrenals, brain and liver; decreased adenosintriphosphoric acid in muscle	473
Lead, Tetraethyl		10, ip., in olive oil	Weakness; nasal discharge; encephalopathy	114

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
Leptazol			See Metrazol	
Liver Concentrate			See Xanthine	
Liver Oil			See Halibut	
Lobelin, Alpha	22-24, ip.		67% blood-sugar rise	664
Luminal			See Phenobarbital	
Magnesium Chlorate, Anhydrous		5250, oral; M/1 sol. 1100, ip.; M/2 sol.; 1-hour tests	Majority died; convulsions; purgation (oral method only)	848
Magnesium Chloride, Anhydrous (See also Chaps. 5 and 6)		2800, oral; M/1 sol. 225, ip.; M/2 sol.	As above	848
	700, subc.; 21-day females	900, same	Maximum safe dose	541
Magnesium Sulfate (Epsom Salt)	400, im.		No visible effects	851
		900; 1300, im.	About LD 25; 100	
	333, ip.		Anesthesia for parathyroidectomy; supplement hourly as needed	434
Exsiccated	700, ip.	1000, ip.	Sleep. Reflexes lost	499
Malachite Green			See Brilliant Green	
Mallophene			See Pyridium	
Mandelate, Ammonium		5000, oral	Minimum toxic dose	565
Mandelic Acid		3000, oral	Minimum toxic dose	565
Manganese Sulfate (See also Chap. 5)	0.01-0.05%, as Mn, in food		Growth promoted	503
	0.009; 0.56, as Mn, per rat, oral; daily		0.009 accelerated, 0.56 retarded growth	524
Mapharsen			See Oxophenarsine	
Mecholyl			See Metacholine	
Menadione (Menaphtone; see also Vitamin K and Chap. 6)	350, oral; daily for 30 days; in 10% acacia	500, same method; young rats	Marked fall in erythrocytes and hemoglobin. Fatal on various days	594
	10, oral; 4-5-week males; daily for 3-11 days; in olive oil		Counteracted hemorrhagic syndrome due to ingestion of various hydroxyquinones; vitamin K superior	765

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Menadione, *cont.*	5-10 per rat, im.; daily for 4 days		Usually lowered blood pressure in induced hypertension	728
Mepacrine			See Quinacrine	
Meperidine (Demerol; Isonipecaine; Dolantin; Pethidine; Eudolat) Hydrochloride	100-200, oral		Usually analgesia	316
	35, subc.		Average analgetic dose	
	43.6, subc.		"Standard Analgesia"	267
		350, subc.	Average fatal dose	239
	50, ip.		Analgesia in some rats	316
	30, ip.		Increased reaction time of tail to heat by 5 seconds	183
	4.6, vein		Same	
		93, ip.; 100-175-Gm. rats	LD 50	316
	10-20, vein		Brief analgesia; convulsions; catalepsy, hematuria and dorsiflexion of tail	239
Mercury Bichloride (Mercuric Chloride; Corrosive Sublimate)		50, oral*	Corrosion; fatal in 24 hours	788
	6-30, oral 5-7, im. 1-4, vein		Immediate hyperlipemia except with oral administration	378
Mercury Oleate (Oleated Mercury)	400 mg. on 29 sq. cm. of clipped dorsal skin; inunction for 2 minutes		With protective cover gave more Hg in the kidney than 7 other official Hg ointments	478
Mersalyl (Salyrgan)	13.6, ip.; 140-240-Gm. males; fasted 18 hours; well hydrated		Marked diuresis after 10 hours; narrow dose range; albuminuria and glycosuria	511
	10-20, im.		Inhibited convulsions from various analeptics	70
Merthiolate, Sodium	45, ip.		Tolerated; antiseptic	553
Metacholine (Mecholyl) Chloride		750, oral 75, subc. 20, vein	LD 50's; cholinergic stimulation	588
Metaphen			See Nitromersol	
Methadon			See Amidon	
Methadren			See Epinephrine, Methyl	

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Methenamine (Hexa-methylene-tetramine; Hexamine; Urotropin)	560, daily, as 0.4% in food for 19 days		Apparently harmless; normal estrus; preliminary lower concentrations were tried	656
Methionine	0.1-1.0% in food; young males		Protected against chronic pyridine poisoning	58 59
	1% in food		Fewer fatalities on repeated exposure to ethylene dichloride	369
	40 per rat; given in special diet		Protected liver against dietary hepatic necrosis	306
	160 per 200-Gm. rat, oral in 5 cc. distilled water		Increased 48-hour survival rate from thermal injury if given 2 hours beforehand	525
Methylene Blue (Methylthionine Chloride)	250-500, oral		Tolerated	21
	50, subc.		Stains cerebellar Purkinje cells	572
•	150, subc		Tolerated	21
		200, subc. 50-100, ip.	Majority died	
Methylrosaniline Chloride (Gentian, Crystal, or Methyl Violet)	250, oral		Tolerated, in vivo antiseptic	19
Methyltestosterone	•		See Androstene	
Metrazol (Leptazol; Cardiazol)	170-180, oral		Stimulation; convulsions	380
	55-60, ip.		Antidoted pentobarbital	314
		100, 150, subc.	LD 67; 100	313
		70-90, ip.	Exhaustion in 2-10 minutes	458
	25, ip.	50, ip.	Minimum convulsive dose. MLD	314
	25, im.		Stomach immobilized and its liquid contents increased	561
	20, vein	50, vein	Convulsions	918
Metycaine (Neothesine) Hydrochloride		1300, subc	Local anesthesia; LD 50	692
		120, ip.	LD 50	
		20, vein	LD 50	
Molybdenate, Ammonium	25 mg., as Mb, per Kg of food		Growth and food intake normal	269
	50, same		Slight growth decrease and toxicity	

Drug	DOSES: Therapeutic or Typical Action	DOSES: Fatal	Remarks	Reference
Morphine	3.5, subc.		About 50% increase in threshold of tail response to heat	841
	20, subc.		Tranquilizing dose	42
	70, subc.; young rats; daily		About normal growth and reproduction	615
	6.8, ip.		Righting reflex depressed	222
	9.1, ip.		Maze response depressed	537
M. Hydrochloride	200-250, oral		Average analgetic dose	239
	1-20, subc.		Pyrexia; catalepsy; exophthalmia	371
	6, subc.		Average analgetic dose	239
	10, ip.		Same	
	3-5, vein		Variable analgesia	
	20, ascending to 200, subc.; daily for 5 weeks		Addiction; weight loss	796
	10, ip., or 2.2, vein		Increased reaction time of tail to heat by 5 seconds	183
M. Sulfate	3 75, subc.		"Standard Analgesia"	267
	40-90, subc.		Hypothermia	371
		119; 280; 285; 253; 220; 172; 196, vein	LD 50's: 14-day; 1-; 2-; 4-; 6-; 12-; 24-month females, respectively	152
		905, oral 572, subc.	LD 50's	329
Morphine, Diacetyl (Heroin)	0.5, subc.		Nearly 50% increase in threshold of tail response to heat	239
	1.1; 1.5, ip.		Righting reflex depressed; maze response depressed	222
"Mustard Gas"			See Dichlorodiethylsulfide	
Myochrysine			See Gold and Sodium Thiomalate	
Naphazoline (Privine) Hydrochloride		385, subc.; 190-250-Gm. males 325, subc; 300-500-Gm. males 50, ip.; same	LD 50's	885

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Naphthylthiourea			See *Alpha*-Naphthylthiourea	
Nembutal			See Pentobarbital	
Neoarsphenamine (Neosalvarsan; Novoarsphenamine)	75-100, subc.		Maximum tolerated	786
	20-40, vein		Trypanocidal dosage	
	200-240, vein	240-300, vein	Maximum tolerated	
		387; 444, vein; slowly	LD 50's: females; males; protected solutions	600 601
		205, same	LD 50; unprotected	
		371, vein	LD 50; whole drug	691
		425, vein	Crystalloid fraction	
		23, vein	Colloid fraction	
Neocinchophen (Novatophan, Tolysin)	400, oral; daily for 2 weeks		Viscera normal	36
	1000; same for 1-3 weeks		Varying visceral necrosis	
	1000, oral; daily		Normal growth and liver; "nontoxic"	37
Neo-Hombreol			See Androstene-ole	
Neonal (Soneryl) Sodium	62.5, subc.	190, subc.	Minimum hypnotic dose	627
	80, subc.	140, subc.	Hypnosis. LD 50; hybrids	399
		135, as the acid, ip.	LD 50	254
Neostam			See Stibamine Glucoside	
Neostibosan			See Ethylstibamine	
Neosynephrine			See Phenylephrine	
Neothesine			See Metycaine	
Nickel Sodium Citrate		20, as Ni, subc.	Paralysis	636
Nicotine	0.05% in food		Estrus stopped; ascribed to semi-starvation	905
	0.4, subc.		Hindleg drag; no adult tolerance	64
		33.5; 35, subc.	LD 60; 90; no tolerance	
		2, subc.; twice daily	Frequent deaths	
	2-2.5, subc.		Convulsive dose	702
	5-7.5, subc ; young rats; daily		Normal growth, decreased activity and fat content	775

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
Nicotine, *cont.*	1-2, subc.; twice daily for 6; 10; 12 months		More nonfertile pairs; fewer litters and fewer young born; progeny less fertile	836
		37; 47, subc.; hybrids	Female; male LD 50; convulsions	399
Nicotine, *d, l, α*, Synthetic		320-640, subc.; 50-Gm. rats		890
Nicotinic Acid (Niacin; see also Chap. 5)	1000, oral; daily for 40 days		Tolerated	852
		7500, oral 5000, subc.	Late stage ataxia, respiratory slowing and cyanosis	
	50; 200, ip.; daily for 24 days		Normal growth; no toxicity	530
As Sodium Nicotinate	3000, oral	5200; 8200, oral	Tolerated. LD 50; 100	589
	5000, subc	7000; 9600, subc.	Same	
		7000, oral 5000, subc.	LD 50's	856
Nicotinamide (Niacinamide)	1000, oral	2700; 3500, oral	Tolerated. LD 50; 100	589
	2000, subc	3500; 4500, subc.	Same	
Nikethamide (Coramine)	360, subc.	470, subc.	Minimum convulsant dose. Average fatal dose	8
	200, subc.	350, subc.	Tolerated. Pulmonary edema; about LD 50	545
	150; 300, subc.		150 antidotal to; 300 synergistic with alcohol	372
	200-300, ip.	Above 450, ip.	Convulsant	458
	95, vein	300, vein	Convulsant	918
Nirvanol (Phenylethylhydantoin)	80, subc.		Racemic; minimum hypnotic dose	782
	80, subc.		Levorotatory	
	100, subc.		Dextrorotatory	
Nitrite, Sodium	20, subc.		Lowered blood pressure	225
		10; 20, subc.	12; 25% methemoglobin formed	62
Nitrobenzene (Oil of Mirbane)		800, subc. 500, ip.	In acacia; methemoglobinemia; convulsions	79

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Nitrofurazone (Furacin)	0.1-0.2% in food, young males		Normal growth; marked leukocytosis	464
		0.4% in food	Cachexia; kidney and liver damage; most die in a week	
		590, oral; 50-100-Gm. rats, 10% suspension in acacia	LD 50; hyperreflexia, tremors, weakness and convulsions	
	20, oral		Cleared blood of trypanosomes in 24-36 hours	207
	100, oral		Complete protection against trypanosomes	
	300, oral		Hyperirritability	
	100-150, oral; every 8 hours for 4-6 days		Slight cloudy swelling of liver	
	3000, subc.		Liver and kidney lesions	
	200-300, ip.	Above 450, ip.	Convulsions	458
	95, vein	300, vein	Convulsions	918
Nitroglycerine			See Glyceryl Trinitrate	
Nitromersol (Metaphen)	30-50, im.		Tolerated; slight pain	786
	4, vein		Slowly; antiseptic	
Nitrous Oxide (Nitrogen Monoxide)	80 vol. % + 20 vol. % air, inhal.		Narcosis; partial pressure 1 atmosphere of N_2O	68
	Same, 2.5 A. P.		Temperature fell to 32° C. in 1 hour	
		Same, 3 A P.	Died in 10 minutes	
Nostal (Noctal)	75, subc.		Narcosis	870
		90, subc	At least 50% acute deaths	
		50, subc or ip.	Many died from delayed pulmonary edema	395
		60, ip.	LD 50	254
	10, subc., twice weekly for 3 weeks		Tolerance to delayed death developed	397 680
Notatin (Penatin; Penicillin B)	0.1-0.3, oral; well hydrated males		Powerful, prolonged antidiuresis	109
	2.5, im, powder or 1 0, subc. in solution		Local edema, swelling, hemorrhage and tissue damage in some	

Drug	DOSES Therapeutic or Typical Action	 Fatal	Remarks	Reference
Novocaine			See Procaine	
Numal			See Alurate	
Nupercaine			See Dibucaine	
Octin (Methyl-iso-octenylamine) Bitartrate		64, ip., as base; 150-250-Gm. males	LD 50	6
Hydrochloride		54, ip , same	LD 50	
Oe			See corresponding words beginning with E	
Optochin			See Ethylhydrocupreine	
Ortal (Hebaral) Sodium	550, oral	2000, oral	Minimum surgical anesthetic dose	320
	100-110; 150, ip.		Same; 150, optimal	320
				315
		140-150, ip.	MLD	320
		240; 265, ip.	About LD 50; 100	315
Ouabain (Strophantin-G)		17; 28; 43, vein	Doses for 10-20; 30-50; 100-200 minutes of infusion; urethane anesthesia	375
		98; 97, subc, 1-month rats; fasted over night; in 0.9% NaCl sol.	LD 50's for females and males, respectively; higher doses with distilled water as solvent; cardiac poisoning	400
		116; 72, same; 2-month rats		
		133; 87, same; 4-5-month rats		
		107; 83, same; 8-11-month rats		
		72; 47, ip.; 4-6-month rats	LD 50 females; males	768
		77.1, vein; 54 mg. per hour; adult males	Amytal anesthesia; artificial respiration; excreted in bile	247
Oxophenarsine (Mapharsen; Metar-	30, oral		Maximum tolerated; useless in trichomoniasis	620

Drug	DOSES: Therapeutic or Typical Action	DOSES: Fatal	Remarks	Reference
sen; Arsenoxide)		22; 24, im.	About LD 40; 60	318
Hydrochloride	0.6, as As, vein		Minimum effective dose in rat trypanosomiasis	869
	3.75, as As, vein		Safe; weakness, restlessness and dyspnea	696
	0.8; 9, vein		Minimum *T. equiperdum* and *rhodesiense* cures	830
	2, vein		Sterilizing; *T equiperdum*	318
		20; 25, vein	LD 20; 80	830
		6; 7.5, as As, vein	LD 70; 100; convulsions	696
Oxygen	Low O_2 tension (10%) or high tension (4 atmospheres), inhal		Depression at 5° C.	39
Oxyquinoline Potassium Bisulfate (Chinosol)		30, subc	Methemoglobinemia; delayed deaths	374
Padutin			See Kallikrein	
Paludrine Monohydrochloride	6-42 (0.5-3 per young rat), oral; 5 days weekly for 20 doses	53-64, oral	All survived; normal growth. 70% mortality in 20 days	149
		58.2, oral	LD 50	149
		75-95, oral; daily	Dead after 1-3 doses; no pathologic lesions	
	80, oral	100-150, oral	LD 0. LD 100	128
		50, oral, same	Normal growth. Growth inhibited; some died; no pathologic changes	149
	30, oral; newly weaned rats; daily for 2 months	33.1, vein	LD 50	
Dihydrochloride	0.0125-0.1% in food for 60 days		Suppression of growth at 0.05%	715
		0.4-0.8% in food	All dead in 6 days due to starvation	
Monoacetate	100, as base, oral	200-400, same	Slight weight loss	
	20, ip. or vein; 100-Gm. rats	40; 60, ip. or vein	LD 0. LD 50; 100; delayed deaths	128
Pantocaine			See Tetracaine	
Pantothenate, Calcium	0.025-0.1 per rat, oral; daily for 4-6 weeks		Prevented adrenal cortex lesions when added to filtrate factor deficient diet	584
	0.1 per rat, oral; six days a week for 60 days		Stimulated growth; prevented graying, but not stippling; same diet	230
	10,000, oral		No toxic symptoms	855

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Pantothenate, Calcium, *cont.*		3400, subc. 820, ip.	LD 50's; respiratory failure in 1-2 hours	
	50, oral; daily for 2 generations		Growth and tissues normal	856
	200, oral; daily for 190 days		Same	
		830, vein	Death in 2 hours	
Pantothenic Acid	0.1 per rat, oral; daily for 6 to 14 days; added to filtrate factor deficient diet		Prevented or repaired adrenal cortex lesions; improved testicular function and skeletal growth	176 27
Papaverine Sulfate or Hydrochloride	300, subc.		Tranquilization and respiratory slowing	786
Para-Amino			See Amino-Para	
Paraldehyde	0.8; 1.0 cc, subc.		Muscular incoordination; deep sleep	589
	0.40-0.45 cc., ip.; 1.1, same	1.35-1.50 cc., ip.	Hypnosis; coma. MLD	499 847
	0.6 cc., rectal	1.5 cc, rectal	Hypnosis. Coma; MLD	847
Parathormone (Parathyroid Extract)	0.1-0.25 cc. per rat, subc., daily		Safe; start with 0.1; cancer rats used	82
	0.4 cc. per rat, subc.; daily		In 3 days marked increase in Ca output	120 219
Paredrinol (Veritol) Sulfate		375-450, subc.; 60-120-Gm rats	D- or l-rotatory about same; circulatory and uterine stimulation	508
Pectin, Beet, Purified	20 per rat, oral, daily for 80 days		Prevented ascites and lengthened life in selenate or selenite poisoning	694
Pelletierine Hydrochloride	900, oral	1000; 1200, oral	Tolerated. LD 50; 100; ataxia; paresis	589
	100, subc.	270, 450, subc.	Same	
Penicillic Acid	20-80, subc.		Good antidiuretic action	613
Penicillin Sodium (See also Notatin)	1000-10,000 units per rat, subc.		Widely distributed in tissues and organs except nervous system	177
	600 Oxford units per rat, subc.; every 2 hours for 9 days		6/14 cures from experimental osteomyelitis	443
	900 units; same, hourly for 9 days		6/13 cured, compared with 1/25 of controls	
	100,000 units, ip.; every 2 hours for 5 doses		No effect on blood urea level	500

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Pentobarbital Sodium (Nembutal; Soluble Pentobarbitone; see also Chap. 2)	50, as acid, oral; males; fasted 20 hours	118, same	Anesthetized half the rats. LD 50	860
		120, subc.		47
	40-50, ip.	120, ip.	Minimum anesthetic dose. Killed majority	821
				136
		75, ip.	LD 50; males more resistant	586
				254
				399
		50, vein; 360-460-Gm. males	MLD	405
Pentothal			See Thiopental	
Perandren			See Androstene-ole Propionate	
Pernoston (Pernocton) Sodium	65, subc.	90, subc.	Minimum narcotic dose	870
		124-130, subc.		47
	50, ip.; females	60-70, ip., females	Tolerated. Delayed deaths; males more resistant	395
				401
Pervitin			See Amphetamine, Methyl	
Pethidine			See Meperidine	
Phanodorn (Phanodorm; Cyclobarbital) Sodium	70, subc.	210; 220, subc.	Minimum anesthetic dose	870
				47
Phemerol Chloride	1/5000; 1/1200 in drinking water; 21-day rats		Poor growth on synthetic diet	831
Phenacetin			See Acetophenetidin	
Phenazone			See Antipyrine	
Phenobarbital Sodium (Luminal Sod.; Soluble Phenobarbitone)	100-110, subc.		Minimum anesthetic dose	627
				870
		140; 200; 210, subc.	MLD; fatal; killed majority	627
				870
				818
	19, ip.		Antidoted strychnine and picrotoxin 22 hours after administration	162
	300-400, ip.		Antidoted strychnine	335
	150, ip.		Outstanding antidote in DDT poisoning	643

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Phenobarbital Sodium, *cont.*	50, as acid, ip.		Increased threshold to electrically induced convulsions	245
		155, as acid, ip.	LD 50	254
Phenol (Carbolic Acid)	Below 1% in drinking water		Normal digestion, absorption and metabolism	356
		300, oral	LD 50	471
		150, oral; in olive oil	LD 50; compare with cresols	193
	77; 217, subc.	657, subc.	No effect; convulsions	215
Phenothiazine (Thiodiphenylamine)	5000, oral*		Maximum tolerated	221
	0.3-0.35% in food for 4 months*		Normal food consumption; decreased growth; 0.35, secondary anemia	837
β-Phenoxy-Ethyl-Dimethyl Dodecyl Ammonium Bromide (PDDB)	10, oral; 5 days a week for 7 weeks		Normal except for somewhat slower growth	51
		800, oral	5/6 survived; marked diarrhea	
		40-45, ip. 18, vein	LD 50's; ascites and shift of fluid with ip. administration	
Phenylephrine (Neosynephrine) Hydrochloride		28, subc.; 190-230-Gm. males 6.8, vein	LD 50	886
Phenylhydrazine Hydrochloride	80, vein		Tolerated; methemoglobinemia and anemia	776
Phosgene (See also Diphosgene)	10 mg. per cubic meter of air, inhal.		Safe concentration or C 0	104
		2200 mg. per minute per cubic meter for 30 minutes, inhal.	Median lethal dose or L (Ct) 50	
		1510-9230, same; 10 min. exposure	Transformed variate rate of dying proportional to dosage	
Phosphate, Sodium		2000, ip	Young rats; convulsions	3
Phosphate, Trimethyl		1.65 cc., oral for 8 days	LD 50; depressed respiration, body weakness, tremors and collapse	194
Phosphorus, Elementary in Oil of Peach Kernels	0.1 per rat, oral; daily up to 68 days		Area of increased density in subepiphyseal region	283

Drug	DOSES Therapeutic or Typical Action	 Fatal	Remarks	Reference
Phthiocol (See also Menadione)	100, oral; daily for 30 days; in 10% acacia	350, same	Marked fall in erythrocytes and hemoglobin. Lethal in a few days	594
Picrotoxin	3.0, subc.	7.0, subc.	Minimum convulsant dose	819
		3.5; 5.9, subc.	Female; male LD 50; adult hybrids	396
		2.9, 5.2 subc	Same; adult albinos	
	4.0, ip.	6.5, 8.0, ip.	Stimulant; tolerated. LD 50; 100	589
	2.0, vein	3.0; 4.0, vein	Convulsant. LD 50; LD 100	
Pilocarpine Nitrate	40; 160, subc		Secretions increased, diarrhea; tolerated	395
		320, 640, subc	Dead after several days; in 1 hour	
Piperazine (Diethylene Diamine)	10-25, oral or ip; twice daily for 2 weeks; cotton rats		Immediate, sustained reduction in microfilariae	377
	25, oral, thrice daily for 30 days		Most effective	
Piperidomethyl-3-benzodioxane	10, ip.		Partial protection of heart against epinephrine	658
Piscidia Erythrina Bark (Fish Poison Extract; see also Erythrina)		2, ip.; dilute alcohol suspension	About LD 50; convulsions	352
Pitressin (Vasopression; *Beta*-Hypophamine)	5 dog pressor units per immature rat; 6 days a week for 15-23 days		Injured tubular epithelium of testes	761
Pituitary, Anterior			See Explanatory Notes and Chapter 12	
Pituitary, Posterior, Extract	0.02-0.06 Voegtlin units, subc.		Males; antidiuresis according to the dose	291
	0.1, vein		Rise in blood pressure	496
		80, vein	U. S. P. Health Service powder used	786
	0.01 U. S. P. unit, subc.		Good antidiuretic effect in 200-Gm. males	863
Pontocaine			See Tetracaine	
Potassium Acetate	1035, ip.		Marked glycogen decrease	760
Potassium Chlorate		7000, oral; M/1 sol. 1500, ip.; M/2 sol.	Average fatal doses; convulsions; respiratory failure	848
Potassium Chloride (See also Chap. 5)		2430, oral; M/1 sol. 825, ip.; M/2 sol.	Same as above	848

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Potassium Chloride, *cont.*	1000, subc.; 21-day females	1200, subc.; same	Maximum tolerated	541
	740, ip.		Marked glycogen decrease	760
$K^{42}Cl$	7.5 millicuries per Kg, ip.; 5.44% aqueous solution		Fairly homogenous distribution in brain in 12-18 hours	164
Praesodynium Nitrate	3.5-4.5, as Pr, vein	Larger doses	Maximum tolerated Death in 3-5 days; gross liver damage	562
Priscol (Benzylimidazoline)	10, ip.		Protected heart against epinephrine	658
Privine			See Naphazoline	
Probarbital (Ipral) Sodium	90, subc.	110, subc.	Ear reflex abolished	627
		110, as acid, ip.	LD 50	254
Procaine (Novocaine; Neocaine) Hydrochloride		1600; 2100, subc.	MLD; LD 50; local anesthesia; convulsions	717 692
	200, ip.	250; 300, ip.	LD 0. LD 50; LD 50	589 692
		564, as base, ip.	LD 50	416
		45-55; 53, vein	Majority died; LD 50	406 692
Progesterone (Lipolutin; Lutocylin; Lutromone; Nalutren;	0.25 per rat, subc.; daily for 3 days		Pseudopregnant, spayed adults: endometrium traumatized; deciduomata developed	28
Progesterol; Progestin;	1.5 per rat, subc.		Inhibited estrus	646
Progestone; Proluton)	2.3 per 19-day male, subc.; daily for 5 days		Heavier ventral prostates	307
	1.0 per spayed rat, subc, 48 hours after 500 units of estrogen		Receptivity to males intensified; synergism	60
(See also Testosterone)		5 per 100-Gm. female, ip., every 30 min. for 3 doses; in peanut oil	Anesthesia in 50%; 1/8 died; males more resistant	744
	0.05 per 27- or 117-Gm. female, subc.; daily for 3 months		Growth rate accelerated more in adults	96
Promizole (4, 2′-Diaminophenyl-5′-thiazolylsulfone)	About 30 daily in food for 10 days		Thyroid iodine reduced; 18% of thiouracil activity	531
	About 10, same		53% of thiouracil activity	

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
Propadrine Hydrochloride		850, subc ; 190-250-Gm. males 380, subc., 380-500-Gm. males 160, ip , same	LD 50's; central nervous stimulation	886
Propylene	40-50 vol. %, inhal.	65 vol. %, inhal.	Anesthesia. Fatal in 2 hours	683
Pteroylglutamic Acid			See Folic Acid	
Pyramidon			See Aminopyrine	
Pyrethrine		2000, oral	In paraffin oil	835
Pyribenzamine			See Tripelennamine	
Pyridine		0.1% in low casein-choline diet fed thrice daily to young males	Lost weight and died in 2-4 weeks; liver and kidney damage; methyl pyridine less toxic	58 59
Pyridium (Mallophene)		270, ip.	Monohydrochloride	879
		560, ip.	Just neutralized	
		520, ip.	Neutralized, stored; depression; late deaths with visceral damage	
Pyridoxine (Vitamin B_6; Adermin; Factor I)	0.01 per young rat, oral; daily		Deficiency dermatitis cured in 10 days; practically normal growth	205 666
	0.05: 0.1 per rat, oral; single dose		Cured 75%; 100% of severe deficiency in 2 weeks	666
		4000, as base, or 5500, as hydrochloride, oral	About LD 50's; tonic convulsions in 24, death in 36-72 hours	853 854
		3100, as base, or 3700, as hydrochloride, subc.	Same	
		658, vein; as hydrochloride	LD 50; convulsions; rapid death	892
Pyrogallol		600-700, subc	Agglutination; blood oxygen reduction	76a
Pyrophosphate, Tetraethyl (TEPP)		1.40, oral 0.65, ip.	Approximate LD 50's; rodenticide	548
Quinacrine (Atabrine; Mepacrine) Hydrochloride	0.01% in low protein diet		Slight toxic effects in females only	260
	0 02%, same		Retarded growth in females only	
		0 04% in food	Life span shortened	

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Quinacrine, *cont.*		0.08% in food	Earlier death on low protein diet; leukocytosis, yellow discoloration; liver, heart and muscle pathology	
	150, oral; 2-3 times weekly		Liver damage in 5 weeks; larger rats more susceptible	758
	45, oral (5% of LD 50); males; 6 days a week for 7 weeks		Growth retarded; hepatic and cardiac damage varied with protein content of diet	756
	45; 90, oral; daily for 12 days		Loss of body weight; severe liver damage; prolonged excretion	734
		900; 670, oral	LD 50	
		615-640, as base, oral	LD 50; toxicity varied with product; given as dihydrochloride	46
	450, oral	450, oral; rats fasted overnight. Same, fasted 36 hours	No fatalities. 22% died; 48% died	736
Quinine Hydrochloride		About 790, subc.; young males	Local necrosis	718
	20%; 40% of MLD, subc.		Normal N excretion; anorexia	
Q. Ethochloride Dihydrate		25-50, ip.	LD 100; respiratory curarelike paralysis	492
Q. Methochloride Dihydrate		60-150, ip.	Fatal range	492
		10, vein	LD 100; respiratory curarelike paralysis	
Rectidon			See Sigmodal	
Resorcinol (See also Heptyl- and Hexylresorcinol)		400-500, subc	Persistent tremors and convulsions	75
Rhenium, as $NaReO_4$	40-230, as Re, subc.; every 2-3 weeks		No hemopoietic stimulation in 2 months	550
	800-850, as Re, ip.	900-1000, as Re, ip.	Immature females; cyanosis, rigidity and tetanic convulsions	
Rhodan			See Thiocyanate	

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Rhodium Chloride	0.25-80, subc.; every 3-5 days		In 4 weeks or more, marked local reaction only	648
Riboflavin (Lactoflavin; Vitamin B_2 or G)	2-2.5 gammas per rat, oral; 6 times a week		Induced 3 Gm. weekly gains in 8-week rats, previously on a 4-week deficiency diet	69
	5 gammas, oral; daily		7.7 Gm. weekly gains	243
	10,000, as such or flavinate, oral		No toxic effects	857
		5000, subc. 560, ip. 790, subc., as Sod. salt 560, ip., same	LD 50's; fatal in 2-5 days by kidney concretions	
	10 per rat, oral; daily		Rats raised through 3 generations	
Ricinoleate, Sodium	400 per rat, oral; fasted 16-24 hours		Almost complete absorption in 24 hours; charcoal passage through intestines retarded	540
	About 500 per rat, in food; daily for 2 months		Almost complete absorption	
Rivanol (Ethodin)		100-120, subc. 40-45, vein	Nephritis; antiseptic dye	813
Rotenone (Tubotoxin; see also Cube Root and Derris)	Up to 500, oral		On lettuce; tolerated	327
		75-100, oral; in ethylene glycol	Light anesthesia; pulmonary edema, gastritis; many delayed deaths	752
		200-1000, oral; in acacia solution	Fatal range; 15-day test	17
Rutin (Vitamin P Substance)	0.25-1.0% in food for 150 days; 90-Gm. rats, or 1% for 400 days		Growth normal at all levels of rutin; estrus, reproduction and tissues all normal	908
	20 mg. pellet implanted in body-wall every 3d day for 12 pellets		Hastened recovery time after irradiation	310
Salicylamide	200, oral; 5 times a week for 13 weeks		No untoward symptoms or tissue changes	422
	1600, oral; 20% suspension in 0.5% tragacanth		Tolerated	
		2000, same	Several died over night; depression	

Drug	DOSES Therapeutic or Typical Action	 Fatal	Remarks	Reference
Salicylamide, *cont.*	250, ip.; 10% suspension in 0.5% tragacanth	500; 1000; 1500, ip.; same	Heavy depression. Some; most; all died; congested, hemorrhagic lungs and meninges	
Salicylate, Sodium	130, oral	1600, oral	Analgesia in 50%. LD 50	383
		400, oral; daily for 21 days	Nephritis and hepatitis	36
		500, same	Majority died in 2 weeks	
		600, same	Fatal within 10 days	
		500-650, subc.	Convulsions; wild rats	875
Salicylic Acid, Acetyl (Aspirin)	500; in food; daily		Critical daily dose for young rats	614
	350, oral	1500, oral; 48-hour test	Analgesia in 50%. LD 50	697
	150, oral or subc ; twice daily		Gastric ulcers developed	201
	200, oral; 5 times a week for 13 weeks		No untoward symptoms or tissue changes	422
		1000; 2000, oral; 20% suspension in 0.5% tragacanth	Some; most died over night; additional delayed deaths	
	1% in 2% alcohol; drinking		Gastric ulcers developed	201
	250, ip.; 10% suspension in 0.5% tragacanth	500; 1000, ip.; same	Tolerated. Some; all died over night; additional delayed deaths; congested, hemorrhagic lungs	422
Salicyluric Acid, Synthetic		3000, subc.*	Convulsions, muscular depression and hyperpnea	345
		1000, vein*		786
Saligenin (Salicaine; Salicyl Alcohol)		About 1000, subc.	Depression; tremors	77
Salmine Sulfate (A Protamine)		100-500 into portal vein; pentobarbitalized rats	Vascular occlusion of liver	748
Salyrgan			See Mersalyl	
Sandoptal	52.5, subc.	175, subc.	Minimum anesthetic dose	627
	65, subc.	160, subc.	Anesthesia	870
Saponin		5, vein	Occasional deaths	805
S. Puriss. (Merck)	1-2, subc. or vein, daily		Anemia of pernicious type in 1-3 weeks	300

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Scilliroside, Amorphous (See also Squill)		About 1.25, im.	Convulsant LD 50	293
		About 25, im.	Cardiac LD 50	
		1.25, vein; mainly females in all 4 cases	LD 50 by slow convulsant action	
		2.5, same	LD 50 by rapid cardiac action	
Scopolamine (Hyoscin) Hydrobromide (See also Atropine)	1.3; 13, as scopolamine, subc.		No depression; slight depression	44
	65-130, same		Marked reflex irritability	
Seconal Sodium	65, oral	125, oral	Minimum anesthetic and fatal doses	820
	60, subc.	140, subc.		
	40, ip.	110, ip.		
	20-25, subc.		Usually decreased oxygen consumption; sometimes excitement and the reverse	642
	51; 42; 42; 34, ip.		Median anesthetic doses for 1½-; 6-; 12-; 24-month rats; both sexes	152
Sedormid	150-200, oral		Hypnosis	196
	400-500, oral	600, oral	Prolonged sleep. Most rats died	
Selenate, Sodium		25-50, as Se, per Kg. of food for 100 days; 4-week rats	Growth impaired and hemoglobin decreased; LD 90	269
		5.25-5.75, as Se, ip.	LD 75 or more within 48 hours	268
		4.0, vein	LD 80-90 in one day	779
Selenite, Sodium		25-50, as Se, per Kg. of food	As for selenate; hematopoietic function disturbed	269
		3.25-3.50, as Se, ip.	LD 75 or more within 48 hours	268
		3.0, vein	LD 40-50; several days	779
Selenium		0.0005-0.0007% in wheat or corn for 2 years; weanling females	Decreased growth rate and appetite; liver tumors	259
		0.001%, same	Most died in 8 weeks; liver cirrhosis; selenide less toxic	
Senna, Alexandria	85.7, powdered; in food; fasted 165-230-Gm. females or 175-300-Gm. males		Laxative dose in 50%; lighter rats were more, heavier less sensitive	865

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
Senna, Alexandria, *cont.*	810, as senna in fluidextract, in food; rats as above		Same; alcohol mostly evaporated before feeding	
Sigmodal (Rectidon) Sodium	50, subc.; females	75-80, subc.; females	Hypnosis; males more resistant	395 399
Snake Venom, Rattlesnake	15-20, ip.	25, ip.	Blood clotted in vivo with large dose	74
Cobra		2, im.		155
Sobisminol			See Bismuthate	
Sodium Chlorate		12,000, oral; M/2 sol. 6000, ip.; M/2 sol.	Average lethal doses; convulsions; respiratory failure	848
Sodium Chloride		8000, oral; M/2 sol. 5000, ip.	Average lethal doses; symptoms as above	848
	3000, subc.; 21-day females	3500, subc.; same	Maximum safe dose	541
Solustibosan			See Stibanose	
Soneryl			See Neonal	
d-Sorbitol	5% in food for 3 generations		No deleterious effects on growth, liver glycogen storing, important metabolic viscera or reproduction	226
Spider Venom, Black Widow, Dried		0.032 per young rat	No pathologic changes	180
Squill, Red, Dry Powder, Coarse		1170, in bait; males; fasted 48 hours 620, same; females; same	Average lethal doses; paresis and convulsions; white squill ineffective by mouth	909
Fine		700, same; males 400, same; females		
Stannic Iodide	150, vein; fresh solution	200, vein, same	Tolerated; no value against staphylococci	462
Stibamine, Ethyl			See Ethylstibamine	
Stibamine Glucoside (Neostam)	40-60, im.; 4 times a week for 2-9 weeks		Adult filariae disappeared promptly, microfilariae slowly	171
	50 per rat, im.		Complex leukocyte changes in trypanosomiasis	423

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Stibanose (Solustibosan)	133 per cotton rat, im.; 6 times a week for 2 weeks		Eradicated filariae	170
Stilbestrol			See Diethylstilbestrol	
Stovaine (Amylocaine) Hydrochloride		25-30, vein	Subc.: local irritation; motor nerves also paralyzed	406
Stovarsol			See Acetarsone	
Streptomycin	300; 900, in food; weanlings; daily for 80 days		Growth slightly retarded; nervous hyperexcitability; tissues normal	596
	160,000-375,000 units/Kg.; in purified food; weanling males; daily for 55 days; 50,000 units, oral, first 2 days		Nutrition good; toxicity slight	232
	580,000-875,000 units, same		Developed biotin deficiency symptoms; marked suppression of intestinal flora, especially coliform count	
	1000 units per 100-Gm. rat; thrice daily for 3 days, or 3000; thrice daily for 4 days		Protected against induced pleuropneumonia and polyarthritis; superior to myochrisin	653
Strontium Acetate		260, vein	About LD 50	189
Strophanthin-G			See Ouabain	
Strophanthin-K		9.4, vein; 0.002% in water	At optimum rate of 100-40 mg./hour; urethane anesthesia; cardiac standstill	563
Alcoholic Extract	10-25, subc.	30-60, same	Tolerated. Fatal range	324 159
Strychnine	3.0, oral	7.5; 15, oral	LD 0, tetanic convulsions. LD 50; 100	589
		6.50; 2.35, oral	LD 50's: males; females	881
S. Sulfate		6.50; 2.60, oral	Same	881
		2.80; 1.45, ip.	Same; greatest sex-variation with oral administration	
		20, oral	Some survived	724
		0.5; 3.0, subc.	MLD at emergence from crawling stage; adult	725
			Samples varied greatly	880

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
S. Salts		1.4; 0.9, as base, ip.	6-week males; females	650
		2.3; 1.1, ip.	18-week males; females	
Succinate, Sodium	250-500, im.		Moderately shortened pentobarbital hypnosis; malonate and glutamate ineffective	71
	1000, im., 15 min. before pentobarbital; large males; fasted 16 hours		Limited prevention of pentobarbital fatalities	647
	1000, im., 15 min. before pentobarbital or amytal		Fewer fatalities and shorter hypnosis; lactate, malate and glucose less effective	792
	6000, im.; given in 6 doses during 2 hours; 24-hour test		No harmful effects	
Succinylsulfathiazole (Sulfasuxidine)	1% in stock diet for 110 days; 2-month males		Prevented ulcerative cecitis	89
		0.75% in food, weanling males	Normal growth first 2 weeks; all died within 34 days	232
	5% in food for 33 days		Normal growth; no toxicity; 4.6 mg.% in blood + 1.4% as sulfathiazole	894
	0.75% in purified ration; young males		Liver calcium increased; less so with sulfathiazole	644
Sulfadiazine	0.125% in food for 30 days		Normal growth; 10.7 mg.% in blood	251
	0.5% in stock diet for 5 months	1% in stock diet for 7 months	Reduced appetite and growth. 60% died; uroliths	689
	0.5% in drinking water or 1% in food for 11-18 days		Thyroid hyperplasia and inhibition	30
	1% in Purina Dog Chow Meal for 2 weeks		Suppressed growth; renal pathology	716
	0.5% in purified ration; young males		Calcium in kidneys increased	644
	300 per 300-Gm. rat, oral; 24 hours after inoculation; daily for 3 days		Effective in Type I pneumonia; sodium bicarbonate harmful	518
S. Sodium		1500, ip.; 8-12-week rats	85% died in 2-4 days	497
		900, ip., daily for 4 days	87% died in 2-5 days	
Sulfadiazine, Dimethyl	300 per rat, oral; 300-Gm. rats; daily for 3 days		Effective in Type I pneumonia	517

Drug	DOSES Therapeutic or Typical Action	Fatal	Remarks	Reference
Sulfaguanidine (Sulfanilylguanidine Monohydrate)	0.5% in purified basal ration; weanlings	2%, same	Retarded growth; better growth with added para-aminobenzoic acid, optimum with liver extract	84
	0.5-3.0% in food for 2 weeks		Thyroid enlargement most marked in immature rats and proportional to concentration in food	539
	2% in food; young rats		Thyroid hyperplasia; food-intake and body-weight declined after 25 days	30
Sulfamerazine (Sulfamethyldiazine)	0.05% in food for 30 days		Decreased growth paralleled decreased appetite; no histopathology	895
	0.5-1% in Purina Dog Chow Meal for 2 weeks		Exceeded 20 mg.% in the blood; suppressed growth	716
	4%, same		Slight to moderate renal pathology	
Sulfamerazine Sodium		1500, ip.	52% died in 1-3 days	497
		900, ip., daily for 4 days	55% died in 3-5 days; less renal obstruction by sulfonamide combinations	
Sulfamethylthiazole	0.5-1.0% in food for 50 days		Stunted growth; urinary concretions; increased neutrophile percentage; atrophy of spleen	877
S. Sodium		500-650, ip.	Urinary concretions	498
Sulfamethazine; (Sulfamezathine Sulfadimethyldiazine)	1000, oral; young rats; daily for 29 days		Prolonged. good blood concentration; kidneys normal	693
	1-2% in Purina Dog Chow Meal for 2 weeks		Exceeded 20 mg.% in the blood; kidneys normal	
	4%, same		No renal pathology	716
Sulfanilamide (Prontylin)	50 per rat; in food; 11-16-month rats; daily		86% survival from surely fatal "pneumonia" or "sniffles"; early treatment best	543
	3000; 3500, oral	10,000; 6250; 3900, oral	Tolerated. LD 50 values; temperature dropped	612 592
	500, oral; daily for 2 weeks		Urine and blood vessels normal	903
	3000, oral; daily for 10 days	4200; 5000, oral; same	Tolerated. LD 50; 100; spleens enlarged	592

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Sulfanilamide, *cont.*	1 per rat in PABA-free food; 40-50-Gm. rats; daily for 10 weeks		Prevented para-aminobenzoic acid deficiency symptoms; PABA better; eliminated disease in colony by feeding it to mothers	555
	200-400, vein; water-suspension		Tolerated	661
Sulfapyridine (Dagenan)	2% in food for 30 days		Decreased growth paralleled degree of anorexia; no histopathology	895
	1.5% in special diet		Anorexia with weight loss; counteracted by raw liver	341
	5000, oral; daily for 10 days		Formation of uroliths	25
	500-1000, oral		Optimal in Type I pneumonia	444
	20,000, oral	30,000, oral	Tolerated. LD 20	591
		2000, oral; daily	Majority died in 91 days; debility; loss of hair	
• S. Sodium	1000, ip.; males	1500, ip.; males	LD 100; alkalinity of salt increased the toxicity	498
Sulfarsphenamine (Sulfarsenobenzene; Sulfarsenol; see also Bismarsen)	16-34, subc. 16-32, vein	400-700, subc. 320-480, vein	Various samples had relatively constant fatal and trypanocidal power; stable 24 hours	867
		590, vein	LD 50	526
Sulfasuxidine			See Succinylsulfathiazole	
Sulfathiazole	0.1-1% in food for 50 days		Decreased growth; urinary concretions; increased neutrophile percentage	877
S. Sodium	500, ip.; males	1250, same	LD 100; urinary concretions; alkalinity of salt increased the toxicity	498
		1100, ip.; 8-12-week rats	64% died in 1-4 days	497
		900, ip.; daily for 4 days	LD 100 in 1-4 days	
Sulfite, Bisodium	0.05% in food		Accelerated growth first 12 weeks	256
	0.1%, same		Impaired growth; toxic	
		0 25%, same	Decreased survival time; polyneuritis and multiple pathologic changes	
Sulfocyanate			See Thiocyanate	

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Sulfurous Acid		0.1 vol. % in air, inhal.	Suffocation; pulmonary edema	264
Synephrine, Meta			See Phenylephrine	
Synephrine, Para (Sympatol)	2-3, vein		Increased uterine tone	7
Synthalin	0.38 per rat, subc ; daily	0.75, same	Safe; growth retarded. Some died, cancer rats	82
	1.0 per rat, subc.		Sublethal; hypoglycemia	
Syntropan		43, vein	LD 50, weak atropine-like action	487
Tannic Acid	10-50, ip.		Water diuresis markedly decreased; not if given subc.	687
Tartrate, Sodium (See also Antimony)	8830, oral		Catharsis; tissues normal	504
	2420, ip.; hooded rats		Weight loss; marked diarrhea; lighter colored liver	
Tellurate, Sodium	25-50, as Te, per Kg. of food		Toxic to growth; alopecia	269
		20-30, as Te, ip.	At least LD 75 within 48 hours	268
Tellurite, Sodium	25-50, as Te, per Kg. of food		More toxic than tellurate	269
		2.25-2.50, as Te, ip.	At least LD 75 within 48 hours	268
Testosterone			See Androstene	
Tetracaine (Amethocaine; Decicaine; Pantocaine; Pontocaine) Hydrochloride	1.5, vein		Convulsant hind leg position in 50%	224
Thallium Acetate	0.12 per rat; in food		Loss of hair	126
T. Sulfate		31, in bait	Anorexia; respiratory failure in 2-3 days	611
Theelin			See Estrone	
Theelol			See Estriol	
Theophylline (Theocin)	2.5-5, oral; rats fasted 16 hours		More potent diuretic than theobromine	332
	20; 40; 80, subc.		Markedly increased activity; superior to caffeine	729

Drug	DOSES Therapeutic or Typical Action	DOSES Fatal	Remarks	Reference
Theophylline, Sodium	100, oral	200, oral	Tolerated. Fatal to some	547
T. and Sodium Acetate	0.10 millimols, oral or subc.; adult males		Marked diuresis and increased chloride excretion	200
Theophylline, Ethylenediamine			See Aminophylline	
Thiamine (Aneurine; Vitamin B_1)	0.1 per young, T. depleted rat, intracardially; daily for 5 days; in 0.9% NaCl sol.		Prompt liver glycogen elevation after medication	814
Thiamine Hydrochloride	3.172 gammas; synthetic, crystalline		Equivalent to 1 international unit by rat growth method	755
	0.8-1.0 mg. per Kg. of B_1-free food		Insured normal growth	26
	4 mg., same		Increased insulin hypoglycemia	118
	6000, oral	9500, oral	LD 0. LD 50	590
	500, subc.	1400, subc.	Same	
	0.250 per rat, im. or ip.; daily for 2-6 days; restricted amount of B_1-free food		Promptly restored impairment of work performance due to deficiency; no benefit in normals	452
		200-300, vein	About same doses for natural vitamin B_1	595
Thiocyanate (Sulfocyanate; Rhodanate) Potassium		854, oral	LD 50	18
		1000, subc.	Paralysis	513
T. Sodium		756, oral	LD 50	18
Thiopental (Pentothal) Sodium	70, oral	190, oral	Anesthetic doses and MLD's	320
	40, ip.	100, ip.		
	25, vein	70, vein		
	40-60, ip.		Decreased 0.2% NaCl solution excretion	759
Thiosulfate (Hyposulfite), Sodium	2500, vein		Maximum tolerated; convulsions	866
Thiouracil	0.02% in drinking water for 10 days; weanling females		Marked thyroid inhibition	28a

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
	0.1%, same for 84-191 days; adult females		After 125 days embryos were resorbed; normal litters on less than 84-day medication	433
	0.1% in food for 14-100 days; young males		Thyroid enlargement; decreased metabolism and growth	292
	0.1% in food; young females		Reduced lactogenic hormone in pituitary	566
	0.2% in food for 3 weeks		Endured low barometric pressure better	298
	0.5% in food		Metabolic depression stationary by 24th day	41
	6.4; in food; daily for 10 days		Thyroid iodine content diminished	532 131
	3.4-4.2 per 300-Gm. rat, oral; daily for 7 days		Thyroid hormone formation and radio-iodine storing decreased; normal after a week	270
	20, oral; 100-200-Gm males; daily for 30 days		Great resistance to alloxan- and pancreatectomy-induced diabetes	413
	80 per older female, oral, daily for 3-4 months		Adrenal cortex involution; urinary thiouracil sediment	57
	0.25, oral; daily for 10-15 days	0.50-4.00, same for 1-16 days	Mild or no symptoms. Cachexia, tremors, convulsions, bloody urine and feces; kidney and liver damage, pulmonary edema	574
	1-4 per rat, subc.; daily; 1st 23 days of life; in peanut oil		Retarded tooth eruption, opening of eyelids and growth	639
	1 per rat, increasing to 4 at end of weaning, then 0.1% in drinking water		Cretinism with continuous administration	417
		350-400, ip.; in 5% acacia	MLD; 6-month females	842
		500, ip.	LD 50	
Thiouracil, 4-Methyl-2-(Thymine)	0.00025% in food during pregnancy only		Transitory infantile light thyroid hyperplasia; growth normal	273
	0.11% in food for 100 days; weanling males		Thyroid hyperplasia; growth and metabolic rate decreased	292
	0.1-20 per rat, oral; daily for 10-240 days		Highly regressive thyroid hyperplasia; single large dose ineffective	274

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
Thiouracil, 4-Methyl-2-, *cont.*	400, oral; young rats; daily for 6 days	600-1200, same	Tolerated. Diarrhea, hematuria and nosebleed	145
		300-320, ip.; in 5% acacia	MLD; 6-month females	842
Thiouracil, 6-n-Propyl (Propacil)	0.1% in drinking water for 10-14 days		Thyroid promptly discharged ingested iodine	861
	0.6-261, in food; 40-80-Gm. rats; daily for 10 days		Most potent antithyroid substance	29
T., 4-n-Propyl	0.002% in food for 4 days; pregnant rats		Entered the fetuses	273
Thiourea	1% in drinking water for 3 months, or 0.5% in food for 12 days; males		Marked increase in resistance to anoxia	485
	1% in drinking water for 4 months		Thyroidectomy-like changes; liver did not lose in weight	486
	0.5% in food for 30 days		Liver glycogen much increased	294
	0.2-0.7% in food		Marked increase in resistance to dichloroethane	369
	0.5% in stock diet for 20-27 days; 150-Gm. males		With decreased weight the organ-weight/body-weight was unchanged	484
	1% in purified food; weanling males		Anemia and leukemia; hemorrhage and necrosis of adrenals	178
	110 per rat, subc., daily for 14 days		Thyroid hyperplasia; more effective than sulfaguanidine	539
In Olive Oil Suspension		44, ip ; stock diet	LD 50's: "Harvard" tame Norways;	203
		640, ip.; Purina Fox Chow		
		4, ip.; stock diet	"Hopkins" tame Norways;	
		1340, same	Wild Norways;	
		1220, same	Wild Alexandrines;	
		1830, ip.; city forage	Wild Norways	
Thorium Chloride		15-20, as Th, vein	Hemoglobin stayed up well	562
Thorotrast (Colloidal Thorium Dioxide)	0.5 cc. per rat, intracardially		Liver and spleen outlined by roentgenograms	802
Thujone, Oil of	2; 4 cc., ip.		Convulsions in 20; in 90%	705
	4 cc., ip.	7; 12 cc., ip.	LD 0. LD 50; 100	
Thyroid, Desiccated	2-3 per rat, oral; daily for several days		Lowered resistance to acetonitril or morphine proportionate to I content	421

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
	0.5-1.0% in food; immature rats; yeast added to diet		Marked inhibition of ovarian development with only moderately stunted growth	241
	300-500 per young rat. in food; daily		Thyroid enlargement	678
	600-800, same		Hyperactivity; learning nearly impossible	
From the Ox	100-500 per rat, oral, daily for several days		Anorexia, myocarditis; liver glycogen decrease	410 411
Thyroxin, Crystalline	0.1 per rat, subc ; daily		Nontoxic to cancer rats	82
	0.5, same		Weight loss in such rats	
	0.025-1.5, same for 5 days		25-69% higher metabolic rate	138
	0 08-0.1 per newborn; subc.		Teeth eruption much accelerated	437
Tin			See Stannic	
Tocopherol, Alpha (Vitamin E)	0.1 per rat, oral; daily		Antisterility requirement in vitamin E depletion	786
	3 per rat, oral		100% littering; females, vitamin-E-free diet	244
T. Acetate	0.9 per rat, oral; daily; in cod-liver oil		More vitamin A stored in liver	31
	0.5-51 per 15-day-suckling of E-deficient mothers, or 13-26 per mother, oral		Retarded postpubertal degeneration of testes; duration proportional to dose	637
	1 per E depleted young male; daily; low protein, E-deficient diet; in olive oil		Prevented testicular atrophy, gastric ulcers and other changes	414
T. Acetate, Butyrate or Propionate	1 per rat, oral		Minimum effective dose	197
Toluol (Toluene)	1 cc., subc.; daily		Slight weight loss; toxic symptoms	56
	0.75-1.25 cc., ip.	1.25-2.0 cc., ip.	50% in olive oil; depression, narcosis	
		1600 parts per million of air; inhal.	Narcosis; death within a few days	
Tolysin			See Neocinchophen	
Trasentine			See Adiphenine	
Tribromoethanol (Avertin; Ethobrom)	200; 400, rectal or subc.	660, rectal	Sleep; reflexes lost	847
		730; 600, subc.	Acute; delayed deaths	

	DOSES			
Drug	Therapeutic or Typical Action	Fatal	Remarks	Reference
Tribromoethanol, *cont.*	135-270, ip.	550, ip.	Sleep; reflexes lost	
In Amylene Hydrate	660, subc.	1400, subc.	Complete sedation	48
(Avertin Fluid)	122; 240-270, ip.	440-550, ip.	Sleep; reflexes lost	499
10% T. in Alcohol	0.85 cc. per 25-Gm. rat, on skin; in 95% alcohol		Anesthesia in 15 minutes	535
Trichloroethanol	200, oral	1000, oral	Minimum hypnotic dose	125
	300; 400, oral		Anesthetic dose; LD 0	593
		625; 800, oral	LD 50; 100	
Trichloroethylene (Chlorylen; Trethylene)		23 mg. per liter of air (4%), inhal.	Fatal in 6 hours	454
		50-135, same for 90 minutes	Fatal range; narcosis; delayed death infrequent	52
Trimethadione (Tridione)	0.25% in food for 9 months		Normal growth	673
	0.5; 1.0%, same		15; 40% less weight gained; cloudy swelling of liver and kidneys	
	500-1000, subc.		Increasing ataxia	
	1500, subc.	2000, subc.	Slept 4-6 hours. LD 50	
	250-500, ip.		Increased threshold to electrically induced convulsions	245
Tripelennamine (Pyribenzamine)	5, oral; daily for 5 months; both sexes		No toxic effects	561a
		570; 515, oral 340; 225, subc. 12; 12, vein	LD 50's, males; females; convulsions; local necrosis at site of injection; sex variation abolished by spaying	
Tropacocaine		15-20, vein	Cocaine-type convulsions	406
Trypan Blue	1000, oral		Tissues stained	19
		300-400, subc. 300-350, ip. 200-300, vein	Lung congestion; of little value in leprosy	
Tryparsamide		750, subc.	MLD	786
	200-813, vein	2000-2750, vein	Trypanocidal dose varied with species	806
Tuamine Sulfate		135, subc.; 190-250-Gm. males	LD 50's	886

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
		160, subc.; 300-500-Gm. males		885
		34, ip., same		
		42, ip.; 150-250-Gm. males	LD 50; calculated as the base	6
Tuamine, Methyl, Hydrochloride		47, same	Same	6
Tubotoxin			See Rotenone	
Tungstate, Ammonium	0.5% as W, in food for 70 days		4-5% weight loss	448
		2%, same	Majority died	
Tungstate, Sodium	0.1%, as W, in food for 70 days		9-11% weight loss	448
		0.5%, same	Majority died	
	0.1%, as W, in food for 100 days; 37-day females		Mainly deposited in bones and spleen; less in skin, liver and kidneys	447
		540, subc.	Gastro-enteritis; central paralysis	67
Tungsten Oxide	0.1%, as W, in food for 70 days		6-7% weight loss; with all 3 W compounds females lost more weight	448
Tutocaine (Butamin) Hydrochloride	14.0, vein		Convulsant hindleg position in 50%	224
Tyramine (Phenolethylamine; Tyrosamine)	10-100; 150-200; subc.		Respiratory volume increased; decreased	38
Tyrocidine	1000, oral		Nontoxic; these and tyrothricin data are based on mice, but are essentially applicable to rats	688
	20, ip.	35-90, ip.	Safe. Fatal range; some died in 3-4 days	
		1.25-25.0, vein	Fatal range; restlessness, depression and hypothermia	
Tyrothricin	1000, oral		Nontoxic; see under Tyrocidine	688
		10-80, ip.	Fatal range; some died in 3-4 days	
		1.2-5.0, vein	Fatal range; restlessness, depression and hypothermia	

Drug	DOSES Therapeutic or Typical Action	 Fatal	Remarks	Reference
Uranium Sodium Tartrate	820, as U, oral		Slight nephritis when killed after 1 month	911
		0.41, as U, subc.	Nephritis; paralysis and loss of weight	
Urea (Carbamide)	73-152, oral; 180-240-Gm. males fasted for 18 hours		Useful standard for diuretic action	511
	750-1000, oral; in 50 cc 0.9% NaCl sol. per Kg. of rat		Good to marked diuresis; more liquid excreted than given	551
Urethane (Ethyl Carbamate)	800-1100, subc. or im		Surgical anesthesia lasting 2-3 hours	589
	780; 1650, ip	1800, ip.	Sleep; reflexes lost	499
	200, ip.		Marked fall in systolic blood pressure	810
	1000, ip.; 10% solution		Inhibition of corneal epithelium mitoses for 8-12 hours; 87% developed lung tumors	325
Vanadium as Sodium Metavanadate	25-50, as V, per Kg. of food		Diarrhea; impaired growth	269
		4-5, as V, ip.	LD 75+ within 48 hours	268
Vanillin	20; in food; daily for 126 days		Normal growth	191
	64, same for 70 days		Retarded growth; systemic damage; same for ethyl vanillin	
		1200-1500, subc. or ip.	Paralysis	78
Veratridine		3.5, ip.; 100-150-Gm. rats	LD 50; salivation; gasping respiration; slow, irregular pulse; body jerkings	569
Veratrine		4.5, ip.; same	Same	569
	0.25 per 200-Gm rat, subc.		Hypertrophy of adrenal cortex in 24 hours; convulsions	804
Veritol			See Paredrinol	
Vinbarbital (Delvinal)	25; 30, oral		Anesthesia in 50; 100%	362
		130, 200, oral; males	LD 50; 100	
	29, ip. 28, ip. 28, ip. 27, ip.; equal number of each sex in all cases	173, ip. 163, ip. 120, ip. 71, ip.	Sodium salt; doses abolished the righting reflex or caused death in 50% at 1, 2, 3-5 and 6-8½ months of age, respectively	404

Drug	DOSES		Remarks	Reference
	Therapeutic or Typical Action	Fatal		
Vinethene (Divinyl or Vinyl Ether; Divinyl Oxide)	3.9 vapor %, inhal.		Anesthetic concentration	361
		0.28 cc per liter of air, inhal. for 3 hours	About LD 50	587
Vital Red	20-45, vein		Growth decreased; pulmonary emboli with 45	289
Vitamins (See also Chaps. 5 and 6)				
Vitamin A			See Carotene; Halibut Oil	
Vitamin B			See Thiamine; Riboflavin; Nicotinic Acid, Nicotinamide; Pantothenic Acid; Pantothenate; Pyridoxine; Aminobenzoic Acid, Para	
Vitamin C See Ascorbic Acid				
Vitamin D			See Calciferol; Cholesterol; Dihydrotachysterol; Ergosterol	
Vitamin E			See Tocopherol	
Vitamin K_1 (2-Methyl-3-phytyl-1, 4-naphthoquinone)	2000, oral, daily for 30 days, in 10% acacia		No demonstrable toxicity; see also Menadione and Phthiocol	594
Vitamin P			See Rutin	
Vonedrine Hydrochloride	170, subc.; 300-500-Gm rats; 26° C.		Body temperature increased for 1 hour; more sensitive at 32° C	886
		850, subc.; 190-500-Gm. rats	LD 50; central stimulation; narrow lethal dose range	886
		540, subc.; 300-500-Gm. rats	LD 50	889
		165, ip.; same	LD 50	885
Xanthine, Sodium	1000, subc.; daily for 2 days		Protected liver against chloroform	616
	100 per rat, subc.		Protected liver against chloroform inhalation effects	53

Drug	DOSES Therapeutic or Typical Action	 Fatal	Remarks	Reference
Xylol	0.75-2.0 cc., ip.	2.0-2.5 cc., ip.	Slow, prolonged narcosis; benzene-type symptoms	56
	1-2 cc., subc.; daily for 10 days		Moderate anemia	
		1600 parts per million of air, inhal.	Narcosis, starvation and death	
Xyloquine (2, 5 Dimethyl Paraquinone)	1-2 per rat, subc.; daily for 3 days; in 0.9% NaCl sol.		Marked blood pressure reduction in hypertensive rats only	272
	25-100; in food; daily for 7 days		Same; some loss in body weight	
Yatren			See Chiniofon	
Yeast, Dried	10 cc. of 15% suspension per Kg., subc.		Consistently produced fever	770
Yohimbine Hydrochloride	1-4 per 60-100-day female, subc.; daily for 35 days		No change in vaginal smears, ovarian weights or number of corpora lutea	812
	1-2 per 60-day female, subc.; daily for 40-101 days		Prolonged estrus	
	3-4, same		Pseudopregnancy; atypical sexual behavior during estrus	277
Yttrium Nitrate		20-30, vein	Hemoglobin stayed up well with 20	562
Zinc Sulfate (See also Chap. 5)	0.15, as Zn, per young rat. oral; daily		Adequate for growth and health	186
	250, as Zn, oral	500, same	Usually tolerated	11
	75-150, as Zn, subc.		Same	

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14

Hematology of the Rat—Methods and Standards*

By

ADOLPH J. CRESKOFF, THOMAS FITZ-HUGH, JR.,
and EDMOND J. FARRIS

COMPARED with human standards the erythron of the adult albino rat may be described as microcytic, hypochromic, and polycythemic. Similarly reticulocytosis, thrombocytosis, leukocytosis, granulocytopenia and relative or absolute lymphocytosis are the rule. Each element shows a wider normal range of variation than the same element in normal human blood.

The total blood volume of the rat is relatively small and the accessible areas for obtaining blood samples are limited. Because of these facts even small frequent bleedings induce anemia and reticulocytosis, and the trauma of repeated sampling produces local inflammation and leukocytosis. Dietary lacks and disease markedly influence the blood picture. It is obvious that careful technic and adequate controls are essential in any hematological study of the rat.

The standards listed below are those of healthy rats of the Wistar strain, inbred for many generations.

OBTAINING THE BLOOD SPECIMEN

The rat is securely held by an assistant, or is rolled tightly in a towel leaving only the tail protruding. Light ether anesthesia may be employed without significant alteration of the red cell and hemoglobin values. The skin over the dorsal tail vein is dry-shaved and the vein punctured or incised. An alternative method is amputation of the tip of the tail. Sluggish flow or rapid clotting can be avoided by first immersing the tail in warm water or by moistening with a saturated solution of sodium citrate. Excessive blood loss must be avoided; between fillings of pipettes bleeding should be controlled by light pressure. A continued ooze may be checked by application of collodion.

To secure normal blood for morphologic examination it is advisable to use quiet animals. For emotional studies the rats are conditioned to the surroundings for the experiments by daily handling for about 7 to 10 days. The

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blood is secured very quickly, without restraining the animal in any manner, by amputation of the tip of the tail.

Larger amounts of blood can be withdrawn by syringe from the jugular and femoral veins, or from the heart. Amounts up to 10 cc. can be so obtained from a large adult rat. (Cardiac puncture, page 417.) Such bleedings, of course, are followed by marked anemia and render the rat useless for further normal blood study for a period of a month or more.

In securing blood samples from fetal or newborn rats speed of manipulation is essential and assistance usually is necessary. Decapitation, or amputation of an extremity provides the bleeding source. The samples can be taken directly or the blood may be allowed to drop into a paraffined watch glass from which a co-worker simultaneously takes the desired samples. The degree of blood dilution by other body fluids is insignificant. Heart puncture of the newborn rat is an alternative but difficult technic.

THE ERYTHROCYTE

Enumeration is carried out by the standard procedure using Hayem's solution as diluent. In the adult rat the count ranges between 7 and 10 million red cells per cu. mm. Twenty-eight adult males averaged 9.27 million; 22 females, 9.42 million; combined average, 9.35 million. The rat erythrocyte is a biconcave disc with little variation in size or shape. Wright or Giemsa stained preparations are excellent for morphologic examination. In such smears the cells are seen to be smaller and less eosinophilic than human cells (Fig. 134). Their diameter averages 6.3 micra. Polychromasia is common, while stippled and nucleated erythrocytes are only occasionally encountered.

The newborn rat shows from 2.5 to 3.5 million erythrocytes per cu. mm. The count falls somewhat during the first days of life then rises steadily until the adult level is reached, at about the fourth month. Fetal erythrocytes number under 3.0 million just before birth; the ten-day fetus has less than 1.0 million red cells per cu. mm. The fetal cell is huge, averaging 10+ micra in diameter, while that of the newborn is somewhat smaller with a mean diameter of 9 micra. Stained smears exhibit marked polychromasia, stippling and anisocytosis. Macrocytosis is striking. Nucleated red cells number 2 to 10% of the total and consist of erythroblasts and (macro-) normoblasts. The newborn rat shows the same erythrocyte picture except that erythroblasts are absent and normoblasts are fewer (1 to 2%).

THE RETICULOCYTE

This immature cell is best demonstrated by the "wet" method of staining. Clean slides are filmed with a saturated solution of brilliant cresyl blue in absolute methyl alcohol and allowed to dry. A small drop of blood on a coverslip is inverted on the filmed slide. This affords an even spread and the cells are sufficiently stained within a few minutes. The count is facilitated by the use of a field-reducing ocular diaphragm and a hand-tally machine. At least 1000 erythrocytes are examined, preferably from 2 or more random fields. The count is reported as a percentage. When a counterstained or permanent spread is desired it may be prepared by mixing at one end of a glass slide one or two drops of blood and an equal amount of 1% brilliant cresyl blue in normal saline, then spreading, drying, counterstaining with Wright's and mounting in the usual manner. Such "dry" preparations are less discriminating than those prepared by the wet technic, in that small particles of reticulum in the older cells are not well demonstrated.

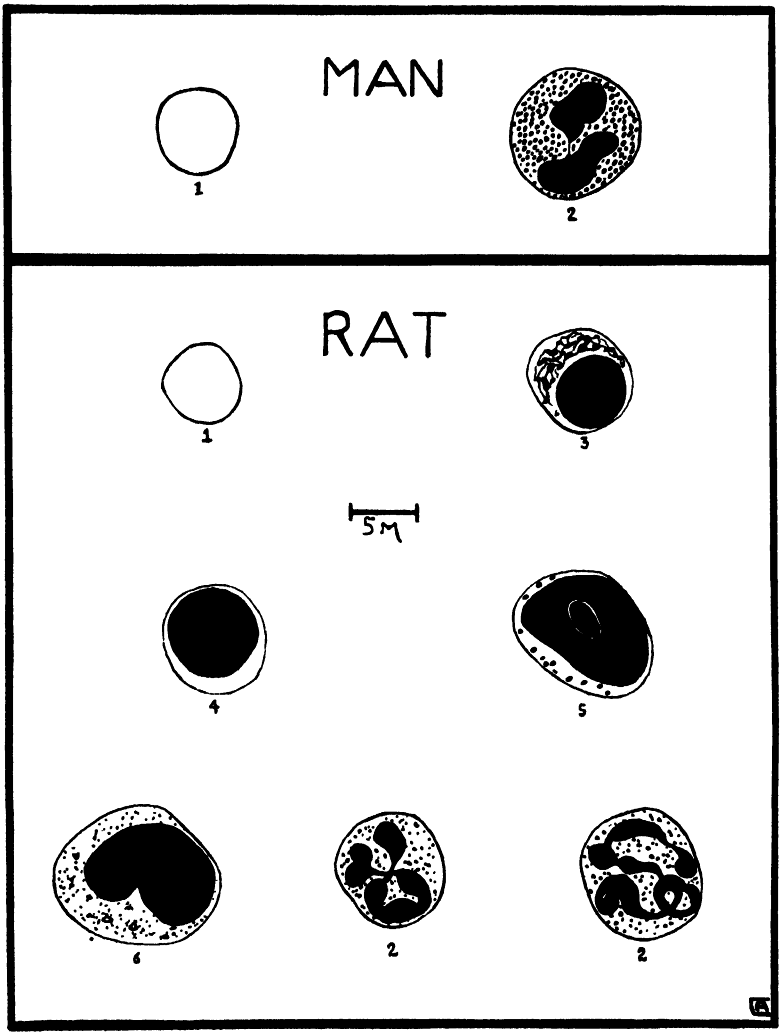

Fig. 134. Schematic Morphology of Rat Blood Cells.
1. Mature erythrocyte. 2 Neutrophilic polymorphonuclear. 3. Reticulated normoblast. 4. Small lymphocyte 5 Large lymphocyte. 6. Monocyte

Fetal reticulocytes require 15 to 30 minutes exposure to the dye before being fully stained. Maximum gentleness in manipulation is necessary owing to the extreme fragility of the fetal erythrocyte.

The stained reticulocyte of the rat is easily identified. Somewhat larger than the mature erythrocyte, it is characterized by the presence of a scroll, skein, filament or granule of dark-blue staining substance. The reticular material may be situated anywhere within the cell borders and the amount present designates the degree of maturity: the younger the cell, the more abundant and compact is the reticular substance. Nucleated and microcytic reticulocytes are sometimes encountered. Since the maturest reticulocytes show only a

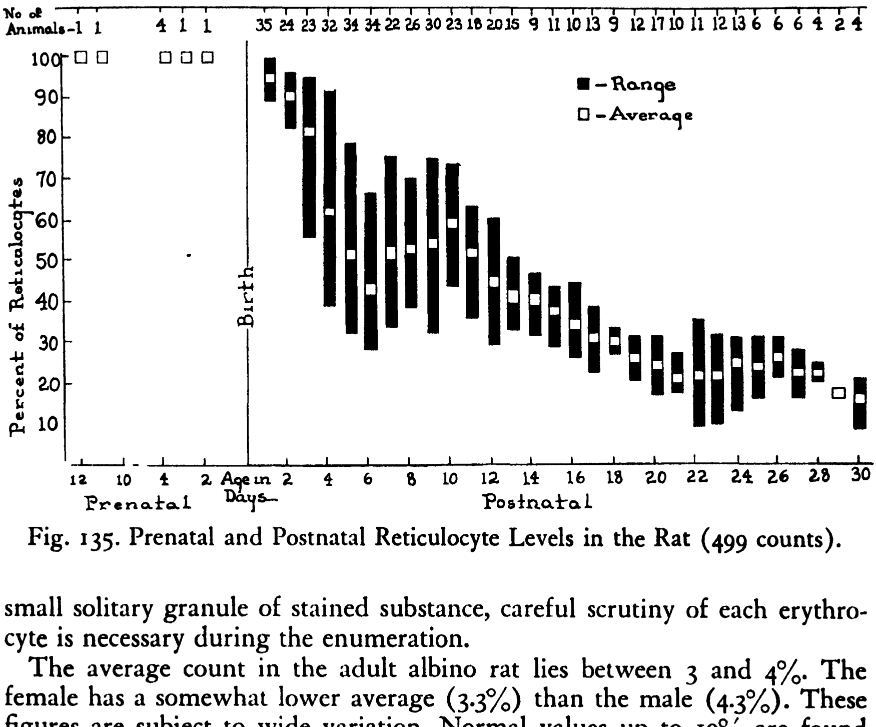

Fig. 135. Prenatal and Postnatal Reticulocyte Levels in the Rat (499 counts).

small solitary granule of stained substance, careful scrutiny of each erythrocyte is necessary during the enumeration.

The average count in the adult albino rat lies between 3 and 4%. The female has a somewhat lower average (3.3%) than the male (4.3%). These figures are subject to wide variation. Normal values up to 10% are found and spontaneous daily fluctuations up to 5% in the same rat are not uncommon. These facts are to be emphasized. There are many experiments in the literature based upon the constancy of the rat reticulocyte percentage. In the writer's opinion a variation of less than ±5% (absolute) is not experimentally significant.

Fetal rat erythrocytes are virtually 100% reticulated, including reticulated normoblasts. Newborn rats exhibit a reticulocytosis averaging 95%. Within the first week of life the percentage drops rapidly, then shows a slight secondary increase, and finally declines slowly and irregularly until the adult level is reached by the end of the third month. The average daily reticulocyte percentage during the first month of life is indicated in Figure 135.

The wide range of reticulocyte values obtained in different nurslings of the same age demonstrates the instability of the reticulocyte level during the first weeks of life. The nature of the curve formed by the daily average figures suggests the possibility that with birth there is some stimulus to increased reticulocyte formation which is manifested between the 7th and 10th days of life.

HEMOGLOBIN

The hemoglobin concentration may be determined by any method requiring only a small blood sample. Estimations performed on a group of 50 adult rats revealed an average value of 101% by the Sahli method (100% = 15.6 Gm. per 100 cc. of blood). Twenty-eight males averaged 99%; 22 females, 103%.

Newborn rats average 75% hemoglobin while late fetal rat blood is somewhat below this figure. After birth there is a slow, irregular increase in hemoglobin concentration toward the adult level. This increase fails to keep pace with that of the erythrocyte so that with maturity the concentration of hemoglobin in the erythrocyte decreases.

Reichert and Brown[17] determined that hemoglobin crystals from the blood of the albino and Norway rat are similar, but the crystals from the blood of the house rat are different.

Abderhalden[1] reported the percentage of hemoglobin is higher in the smaller, younger rats than in the larger, older ones.

CELL VOLUME, COLOR AND VOLUME INDEXES

The Van Allen hematocrit tube is a suitable instrument for determinations of red-cell volume in the rat. Sodium oxalate solution, 1.1%, is a satisfactory anticoagulant. The cell volume determined by this method in 28 adult male rats averaged 49 cc. per 100 cc. of blood; in 2 females, 52 cc. per 100 cc. of blood. The combined average may be taken as 50 cc. per 100 cc. of blood.

"Rat Standards" for color and volume indexes may be established from the above values. It is erroneous to use the standards for man; based on these, the normal rat presents a microcytic, hypochromic polycythemia. If, for the rat, 9.0 million erythrocytes per cu. mm., 100% hemoglobin, and hematocrit reading of 50 are established as 100% normalcy, then color and volume indexes (as well as saturation index, mean corpuscular volume, etc.) may be calculated. For example, given R.B.C. = 7.5 million, Hgb. = 88%, Hematocrit = 41. Then R.B.C. = 83% (of 9.0 million) and Cell Volume = 88% (of 50). Color Index = 1.06. Volume Index = 1.06.

In the newborn rat the average hematocrit reading is 36 and the Volume Index 1.8 (Rat Standards). At the 18th day of life the Volume Index has fallen to 1.02. Other investigators have found even higher hematocrit figures and indexes in the newborn rat. The macrocytosis and high corpuscular hemoglobin concentration of the fetus' and newborn's erythrocytes have been observed by many workers in various animal species.

Table XXI summarizes the average values of the rat erythron.

TABLE XXI

Average values of rat erythron

	R.B.C. millions per cu. mm.	Hgb. % 100 = 15.6 Gm.	RETIC. %	CELL VOL cu. mm. per 100 cc blood	CELL DIAM. micra	NUC. R.B.C. %
Adult Male	9.27	99	4.3	49	6.3	Few
Adult Female	9.42	103	3.3	52	6.3	Few
Adult Aver.	9.35	100	3.8	50	6.3	Few
New-born	2.5–3.5	75	95	36	9	1–2
Fetus	1.0–3.0	<70	100	..	10+	2–10

THE LEUKOCYTE

Enumeration is performed by the standard method. Acetic acid, 2%, to which are added a few crystals of gentian violet, serves as diluent. Smears for morphologic study are made on slides or coverslips and stained with Wright or Giemsa stain. A correction for nucleated red-cell content is necessary when these cells are numerous. This is done by calculating the normoblast total from the total erythrocyte count, or by eliminating nucleated erythrocytes from the leukocyte count by direct inspection in the counting chamber.

The leukocytes of the rat show no important difference in number or differential formula between the sexes. The adult rat averages 9,000 leukocytes per cu. mm., with a normal range of 6,000 to 18,000. In a series of 50 differential counts the following average figures were obtained:

	%	Range
Neutrophils Non-filament	7	2–14
Neutrophils Filament	13	6–25
Lymphocytes	78	55–96
Monocytes	<1	0–3
Eosinophils	2	0–4
Basophils	<1	0–0

The leukocytes of the rat, conditioned for emotional studies, show important differences in number and differential formula. The male rat of 252 days averages 14,000 leukocytes per cu. mm. with a normal range of 8,800 through 19,000. The female rat of 125 days averages 9,700 leukocytes per cu. mm. with a normal range of 6,800 through 15,725. In a series of 24 differential counts the following average figures were obtained:

	%	Range
Polymorphonuclears or neutrophils	35	24–47
Lymphocytes	60	47–73
Monocytes	2	0–3
Eosinophils	2	0–7
Basophils	1	0–1

The differences in the leukocyte counts reported above may be accounted for by the fact that emotional stimulation caused by restraint of the animal in a towel or general handling caused the change in blood picture. It was shown by Farris,[6] that emotional stimulation of short duration produced a marked relative lymphocytosis in the normal albino rat, with a 12.08% increase in the lymphocytes. It should also be noted that a struggle of a relatively few seconds may produce an immediate leukocytosis.

The granulocytes are characterized by a serpentine, coiled, or twisted nucleus. Nuclear lobulation is not pronounced. Cytoplasmic granules are relatively few but stain characteristically. Lymphocytes exist in both large and small forms. The nucleus of the large lymphocyte exhibits a typical central nucleolus, indicating immaturity. The monocyte is the largest of the series, possessing abundant cytoplasm which includes a few azurophil granules and a kidney-shaped nucleus (Fig. 134). The monocyte and lymphocyte do not take the oxidase stain. In a small series of observations 70% of the leukocytes were found to be oxidase-negative.

The leukocytes of the newborn rat average 3,200 per cu. mm. with a differential formula as follows:

Myelocytes	25%
Metamyelocytes	25%
Granulocytes	25%
Lymphocytes	25%

The adult level of the leukocyte count and its components is reached during the 3rd week of life.

Leukopenia. Investigation (Farris[6a]) has shown that there is a leukopenia in the female albino rat at the peak of estrus, about 6,000 leukocytes per cu. mm., at a time when activity of the animal would lead one to expect a leukocytosis. During the diestrous interval there is a return to the normal leukocyte count of about 10,000 per cu. mm.

MISCELLANEOUS STANDARDS

Thrombocytes. Platelets are counted with Rees and Ecker's[16] fluid as diluent.

Sodium citrate	3.8 Gm.
Formalin	0.2 cu.mm.
Brilliant Cresyl Blue	0.1 Gm.
Distilled Water	100.0 cu.mm.

(Filter before using)

Using this fluid, blood is drawn and diluted as for the erythrocyte count. Speed of manipulation is necessary to prevent clumping and disintegration of the platelets. After mixing, the counting chamber is filled and the platelets allowed to settle for ten minutes before the count is begun. Under the high power of the microscope the platelets appear as small (0.5 micron) blue-stained bodies lying between erythrocytes.

The platelet count in the adult rat averages 800,000 per cu. mm. with a normal range of 500,000 to 1,000,000. In the newborn rat the average count is 400,000 per cu. mm.

Resistance of the Erythrocyte. Using standard methods, beginning hemolysis is noted at 0.475% saline solution. Complete hemolysis occurs at a concentration of 0.300% saline. There is no difference in erythrocyte resistance between the sexes.

Coagulation Time. Blood is allowed to enter a finely drawn capillary tube of uniform bore. At 15-second intervals small pieces of the capillary are broken off. When a strand of fibrin is seen to join the broken ends of the capillary, coagulation is deemed complete. The time elapsing between the first flow of blood and the appearance of the fibrin thread constitutes the coagulation time. The average time for the white rat is 2½ minutes. No sex difference is apparent.

Ingle and Corwin[9] determined coagulation time by means of a Boggs' coagulometer to be an average of 138.0 ± 6 seconds.

Clot Retraction. Into a small saline-rinsed test tube are placed approximately 2 cc. of blood. At room temperature clotting occurs within three minutes and retraction of the clot begins within ten minutes. At the end of sixty minutes retraction is complete. The clot is somewhat friable but cohesive.

Bleeding Time. This may be determined by snipping small wedges from the periphery of the ears. By this method the bleeding time averages two minutes.

Blood Volume. A method for following blood-volume changes of the white rat has been described by Cartland and Koch.[3] The blood volume per 100 Gm. body weight is reported at 6.7 cc.

Specific Gravity. The specific gravity of the blood of the rat is 1.056 (Sherrington and Copeman[18]).

Percentage of Water. The percentage of water in the entire blood has been determined by Hatai[8] for the albino rat. Blood was secured by chloroforming the rat, exposing the heart, clipping the tip away, and catching the blood in small glass weighing bottles. Fresh weight was taken immediately, and after drying the residue the weight was obtained. The results are herewith listed:

Age, Days	Water, %	Age, Days	Water, %
0	96	250	91.6
50	93.2	300	91.5
100	92	350	91.4
150	91.8	400	91.3
200	91.7	450	91.2

Sedimentation of Erythrocytes. Cutler tube determinations carried out in a small series of rats showed the following sedimentation rates:

	60 min	120 min
	mm	mm
Male	0.7	2.0
Female	1.8	2.8

Since this method requires 4.5 cc. of blood the rat is rendered anemic following the test. Wintrobe's method, using 1 cc. of blood, may be employed, but the loss of even this quantity of blood produces anemia in the rat.

The Bone Marrow. Marrow sections may be prepared according to the method described by Custer.[4] Femoral marrow is most suitable for study. Marrow smears are easily prepared by drawing the specimen over a glass slide in serpentine fashion and staining with Wright's, Giemsa's or May-Gruenwald-Giemsa stain. Such preparations exhibit excellent cellular detail and are suitable for differential counts.

The bone marrow of the rat is similar to that of man but is more hyperplastic. It is preponderantly erythroblastic, pronormoblastic, and normoblastic. During early life the marrow is highly cellular (60-90%), becoming less cellular (30%) after the first month of life. Data on the leukocytic components of rat marrow are not available.

Bartonella Muris. It has been observed that removal of the spleen in the adult albino rat is followed by severe anemia within a few days to three weeks, a condition described as Bartonella muris. This anemia (Marmorston-Gottesman and Perla[13]) is characterized by a drop in the normal red-cell count from 8 to 10 million cells per cu. mm. to about 1 million. There is also a corresponding drop in hemoglobin. In the red blood cell appear inclusion bodies known as Bartonella muris bodies. These resemble diplococci and rods on the surface of the cell, and occur in large numbers (20 to 30%) at the height of the anemia. About 20% of the rats die within 8 to 10 days from anemia. The anemia begins as early as the 3rd day and as late as the 16th day, reaches a peak in about the 8th to 12th day, then diminishes. In the circulating blood are present poikilocytes, anisocytosis, polychromasia, and normoblasts. It should be noted that many laboratories report this condition in purebred rats. It is known that The Wistar Institute stock is free of this condition (Farris, unpublished communication), although albino rats claimed to be from original Wistar Stock and bred in other laboratories have been reported to have Bartonella muris.

Nutritional Anemia. Elvehjem and Kemmerer describe a technic for the rapid production of nutritional anemia in rats.[5] These rats are apparently suitable for iron, copper and manganese metabolism studies.

The procedure is essentially as follows: Litters of rats are weaned between three and four weeks of age, in cages with wire-screen bottoms, and fed whole cow's milk ad libitum until distinctly anemic, usually in six to eight weeks, or two weeks after weaning. The mother is removed to a separate cage for dry-ration feeding, dusted clean of food particles, then returned to the young. After 12 days of age the young consume some of the cow's milk, but no other food. By 17 days of age they consume definite amounts of cow's milk, and at 21 days of age they are removed from the mother, and a severe anemia develops within two weeks after weaning. Few rats survive longer than four weeks.

Experimental Polycythemia. Marshall[14] has shown that a true polycythemia could be produced in rats by the administration of a milk diet supplemented by salts of cobalt, iron, copper, and manganese.

The red-blood-cell count of blood obtained by heart puncture revealed a polycythemia of from 10 to 13 million cells per cu. mm., in contrast to about 8 million for the normal red control.

BLOOD GROUPS OF THE RAT

By Clyde E. Keeler

Like so many mammals that have been studied, the rat, Mus norvegicus, is known to have upon its red blood cells, agglutinogens capable of clumping these cells together when they are brought in contact with an appropriate serum containing the corresponding antibodies.

An extensive study by Burhoe has revealed a natural agglutinogen A in the erythrocytes of certain individuals and a corresponding agglutinin, "a," in the blood serum of rats lacking A. In addition there was found an immune agglutinogen B on the erythrocytes of certain other rats. Whereas the corresponding agglutinin was not found in rats lacking B, these animals

TABLE XXII

Blood groups of rats

Description	Source	Blood Group
Red-eyed non-agouti (cream) hooded	Horace W. Feldman Univ. of Michigan	O
Ruby-eyed	Horace W. Feldman	AB
Curly$_1$	Helen D. King The Wistar Institute	AB
Curly$_2$	Paul W. Gregory Univ. of California	AB
Self black (some carrying kinky and Irish)	Horace W. Feldman	A
Blue-hairless	Elmer Roberts Univ. of Illinois	AB
Black-hooded (not inbred)	Helen B. Pugh South Hadley, Mass.	AB and AaBb
Albino	Burton Carmichael University of Maryland	A
Hairless	Elmer Roberts	AB
Albino (not closely inbred)	Charles Lee U. S. Bureau of Fisheries College Park, Maryland	A and AB
Black-hooded	Harold Morris U.S.D.A., Washington, D. C.	AB
Pink-eyed yellow	Horace W. Feldman	AB
Albino	Mass. State Antitoxin Laboratory Forest Hills, Mass.	A
Wild Rats	Caught at Forest Hills, Mass. and at College Park, Maryland	AB
Albino Wistar Strain	The Wistar Institute	A
Albino Experimental Strain	The Wistar Institute	A
Cinnamon	Helen D. King	O, A, and AB
Mutant Albino	Helen D. King	A, B, and AB

by an immunity reaction produced "b" antibodies in their serum when injected with red cells bearing B.

Four serological types of rats are known as regards their blood groups, namely, those possessing O (that is, neither A nor B), those possessing A, those possessing B, and those possessing both A and B. Linkage studies completed by Burhoe make it plain that A and B specificities of rats are independently inherited rather than being due to a pair of allelomorphs or alternative forms of the same gene. Linkage tests have shown that the genes underlying both A and B reside in chromosomes other than those in which reside the genes producing the characters of non-agouti, curly$_1$, curly$_2$, dilute, hairless, hooded, kinky, red-eyed yellow.

For the benefit of those wishing to know the blood formula of rats for blood group studies or other purposes, a survey table by Burhoe is published in Table XXII.

It is interesting to note that only one strain (Red-eyed non-agouti cream-hooded) lacks both A and B agglutinogens. It is also singular that all strains of albinos tested are apparently purebred for A, suggesting that all albino strains are related, whereas only one non-albino strain (self black, some carrying kinky and Irish) has this formula. Since Dr. King's curly$_1$ strain was derived from a wild strain it is not surprising that its formula is AB.

Blood for blood grouping tests is procured by cardiac puncture of the etherized donor. This technic may be perfected to such a degree that it causes no detriment to the animal during a series of bleedings extending over a number of weeks even in females suckling young. The method is described in the following section.

Diagnostic sera containing "a" is present as a normal constituent of blood in rats lacking A on their erythrocytes. The normal titre is such that it produces positive reactions at dilutions of 1/8 to 1/128 to be used in diagnosing rats over six weeks of age. For determining the formula as regards A of rats under six weeks of age an increase in titre may be produced in some cases by a single intraperitoneal injection of whole blood (2.5 cc. to 5.0 cc.), the increase in strength reaching its peak (256-2048) about the seventh day thereafter.

Serum diagnostic for B, that is, containing isohemagglutin "b," is not normally found in rats but may be produced by injections (.5 cc. to 5.0 cc.) of whole blood of rats containing B into animals lacking B. The titre of "b" suitable for diagnostic purposes must be maintained by semi-weekly subcutaneous or intraperitoneal injections.

The actual blood grouping tests are made by mixing on a slide one drop of physiologic saline solution and one drop of diagnostic serum. The slide may be rocked from time to time and allowed to stand for fifteen minutes before a final reading is taken under the microscope.

Only titres of 1024 and over may be used in diagnosing B in the newborn rat, whereas no serum has been produced by Burhoe capable of diagnosing A in the newborn.

At birth the agglutinophylic capacity of rat erythrocytes is low for both A and B, this ability rapidly developing during its first two weeks. During the second month it reaches its adult level which is maintained throughout life.

In Figures 136-139 are shown Burhoe's methods of withdrawal and injection of blood for the purpose of building up antibody titres of the diagnostic isoagglutinins in rats.

Drawing Blood from the Rat Heart. Burhoe's[10] method of drawing blood from the rat heart is similar to the procedures commonly employed with guinea pigs and rabbits.

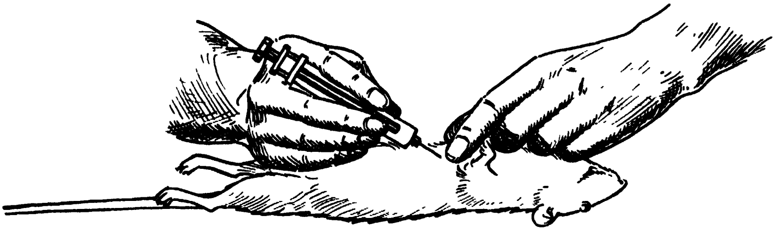

Fig. 136. Locating Heart.

The rat is anesthetized in an etherization jar until completely limp.

A small towel is laid on the operating table and some shredded tissue is arranged on the towel to form a trough. The etherized rat is placed on its back in the trough and a small bottle containing a few drops of ether is disposed sidewise on the table so that the mouth of the bottle loosely surrounds the rat's nose to insure continued anesthesia.

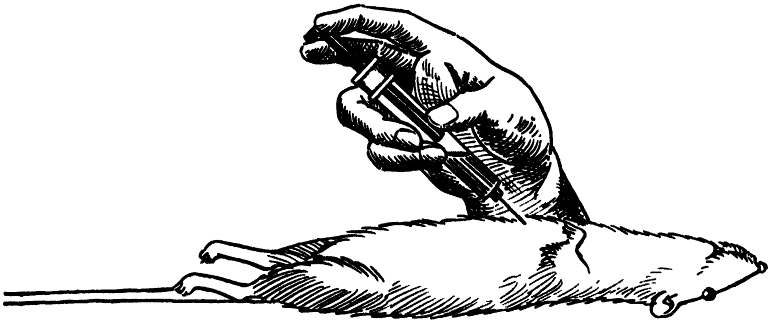

Fig. 137. Withdrawing Blood.

Care must be taken not to over-etherize the animal, but merely to maintain it in a state of surgical anesthesia by approaching and withdrawing the ether bottle as physical signs indicate.

During the operation the rat lies with its head to the operator's left. With the index finger and thumb of the left hand (thumb on the rat's right side), the operator locates the heart as the point of maximum palpitation beneath the 4th, 5th, and 6th ribs.

For drawing the blood one employs a sterile 2 or 5 cc. Luer type glass syringe with gauge-26 needle, ½ inch long, rinsed in and out with sterile sodium-citrate-salt solution (1% sodium citrate in physiologic saline solution) so that the plunger works freely. (A ¾-inch needle length may be found necessary for the largest rats.)

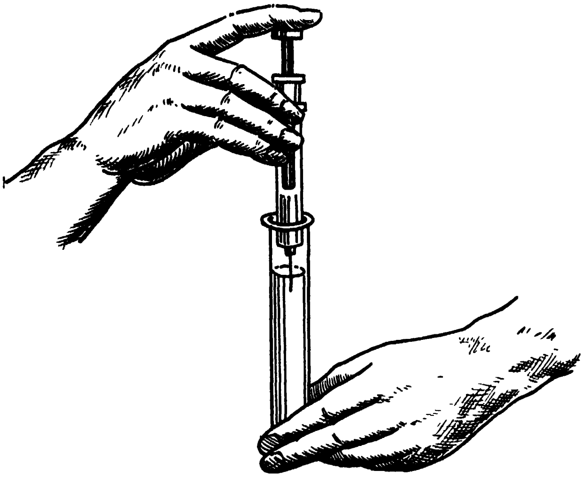

Fig. 138. Diluting the Blood.

When the heart is located a few drops of 75% alcohol are poured on the surface of the left side above the heart as a sterilizing precaution. The barrel of the syringe is held between the thumb and the 4th and 5th fingers of the right hand, the plunger being grasped between the index and middle fingers.

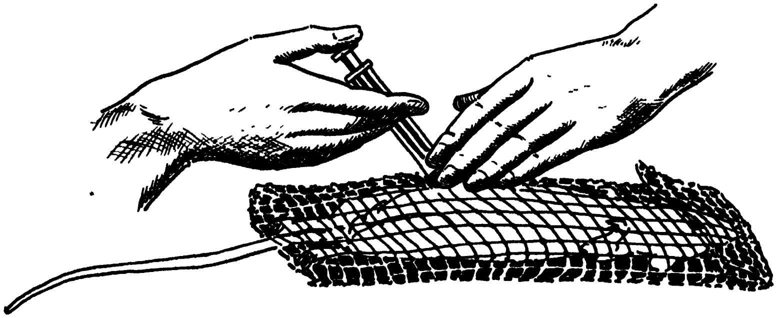

Fig. 139. Injecting the Recipient.

With the syringe in this position the needle is slowly pushed forward into the thoracic cavity toward the point of throb at an angle of about 45° from the long axis of the rat's body.

When the heart may be felt throbbing against the point of the needle, the syringe may be pushed into it. If the right ventricle is penetrated the blood flows somewhat slowly, but if the left ventricle is pierced the blood spurts into the syringe. As blood flows in, the plunger is gradually drawn out to provide more blood space in the syringe.

If an auricle is punctured the blood flows evenly. If the tip of the ventricle only has been pierced, and consequently blood does not flow, this fact is indicated by a tugging at the tip of the needle. In this latter event the needle is withdrawn from the heart and reinserted a little more anteriorly. One little spurt only may indicate that the heart or a larger vessel has been completely transpierced.

Burhoe reports that by use of this method 5 cc. of blood may be drawn from a rat in about 20 seconds, but that his average time is 40-60 seconds. Quantities of blood up to 4% of the body weight have been withdrawn without serious effects.

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15

Radiologic Considerations

By

E. P. PENDERGRASS and J. Q. GRIFFITH, JR.

With Sections by Harry Shay and
J. Gershon-Cohen

TECHNICS OF EXAMINATION

In recent years the rat has been employed with increasing frequency in studies of nutrition and metabolism. Such studies have, at times, involved the taking of roentgenograms. Moreover, as suggested elsewhere in this text, the rat should be used more and more in general physiologic experimentation. For these reasons it seems worthwhile to describe a number of roentgenologic technics and to discuss certain studies in radiation treatment which may be useful in themselves but which also may serve to indicate the potentialities of the rat for this type of investigation.

The rat is a comparatively small, thin animal. When only a part is to be examined, a dental or occlusal film may be adequate. Satisfactory exposures may be obtained with the use of a portable x-ray apparatus. Thus desirable economies in film and equipment may be practiced.

In many instances the rat, under anesthesia, may be simply placed upon the film. Sometimes, when it is desirable to maintain the animal in a "spread-eagle" position, the following technic has been found satisfactory. A circle or square is outlined with sturdy wire. Stitches of linen thread are placed in the skin of the extremities, the ends carried to the wire frame, and fixed with adhesive.

In the roentgenographic illustrations (Figs. 140-162) details of exposures are not given, as there are so many variable factors with different machines. Details of the preparation of the animal are given in the legend for each figure, and in certain instances are amplified in the appropriate portions of the text.

In addition to the roentgenograms described here, the reader should be referred to the work of Menville and Ané.[1] They injected thorotrast into the peritoneal cavity of rats and studied its absorption through the lymphatic system of the diaphragm and into the lymph nodes and thoracic ducts of the thorax. Mortimer[2] secured remarkable roentgenograms of the skull in rats using a film with a fine grain emulsion, such as is used in miniature cameras.

The factors employed were approximately 40 KV, 15 M.A., and an exposure of 8 seconds.

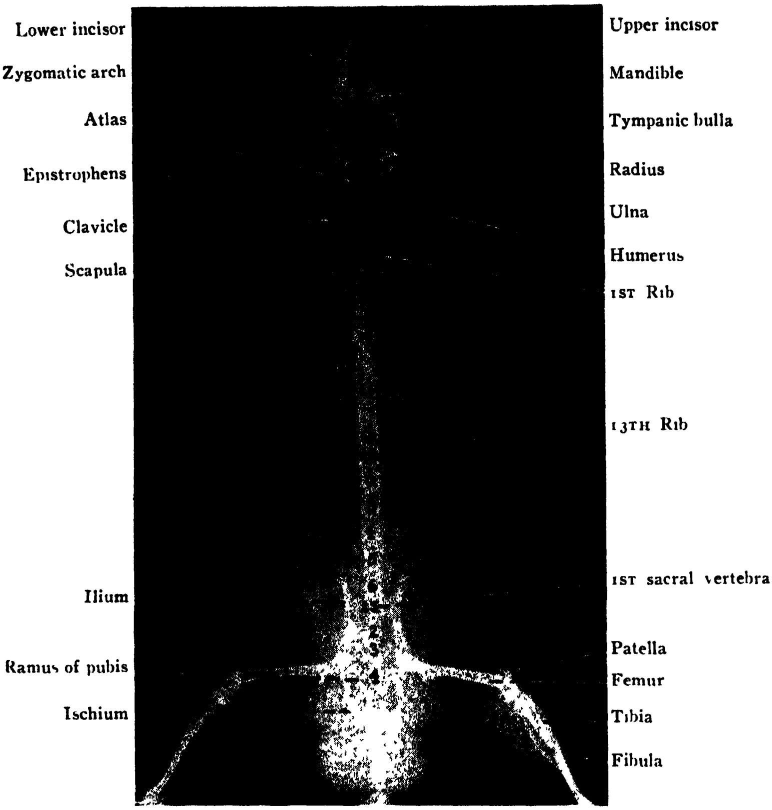

Fig. 140. Roentgenogram of Normal Rat Showing Skeleton, Anteroposterior View.

EXPERIMENTAL RADIATION

Experiments involving irradiation of rats fall into two general classes: 1. Those in which the rat is a biologic test tube for measuring x-ray effects; 2. Those in which the x-rays are used to produce an effect on a tissue or organ. This effect is usually one of destruction. In such experiments the primary purpose is to study the physiologic effect of the alteration in function of the tissue or organ, and the x-rays are simply the means of producing such changes. Of course, certain experiments fall into both classes.

The biologic effect of x-rays has been studied: (1) On virgin female rats, by Murphy[8] by using radium and employing graded doses of millicurie hours. He found that 14% of animals receiving less than 450 millicurie hours were sterile as were 74% of those receiving over 450 millicurie hours. In the offspring he occasionally found clubbed feet or absent toes.

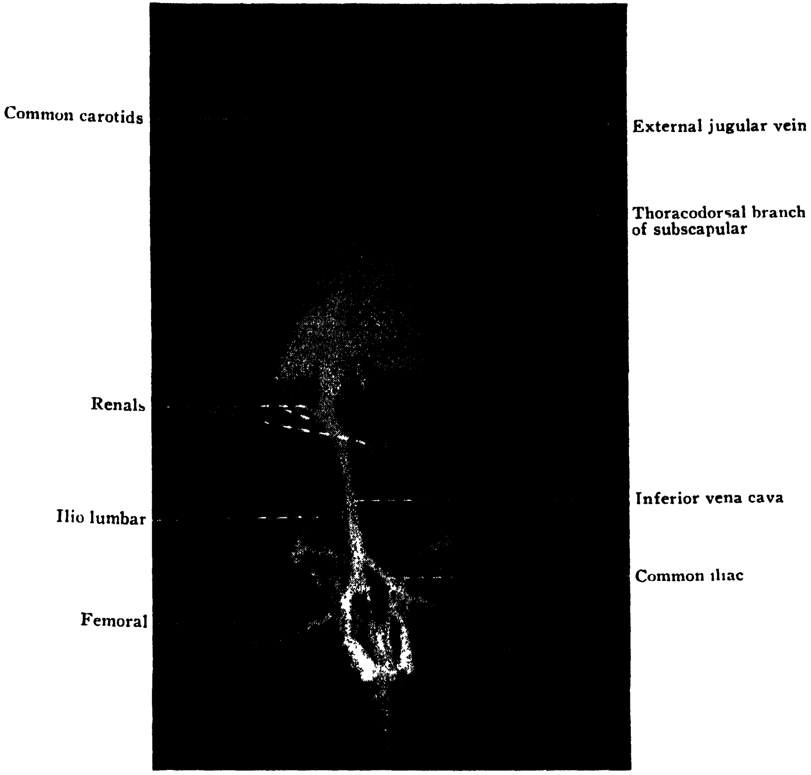

Fig. 141. Angiogram of Living Female Rat. Two cubic centimeters of thorotrast were injected slowly into the left external jugular vein, which appears distended. About four minutes later the roentgenogram was made.

Fig. 142. Angiogram of the left upper extremity of a normal rat, obtained by injecting 1 cc. of thorotrast into left subclavian artery immediately after death.

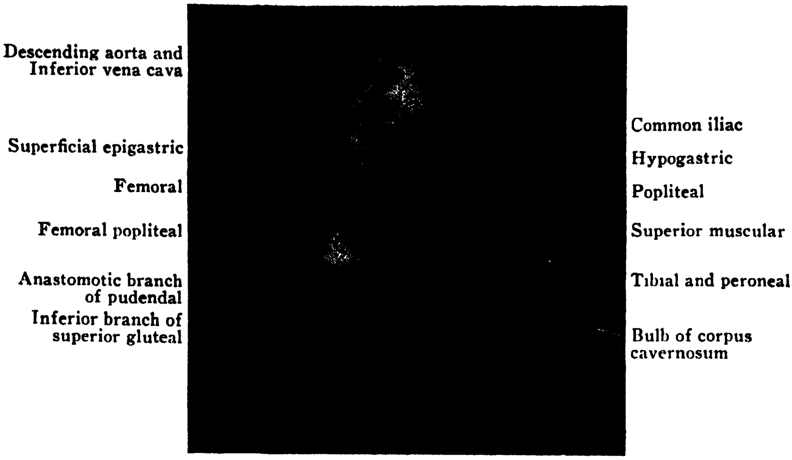
Descending aorta and Inferior vena cava
Superficial epigastric
Femoral
Femoral popliteal
Anastomotic branch of pudendal
Inferior branch of superior gluteal
Common iliac
Hypogastric
Popliteal
Superior muscular
Tibial and peroneal
Bulb of corpus cavernosum

Fig. 143. Angiogram of the lower extremity of a normal rat, obtained by injecting 1 cc. of thorotrast into the abdominal aorta immediately after death.

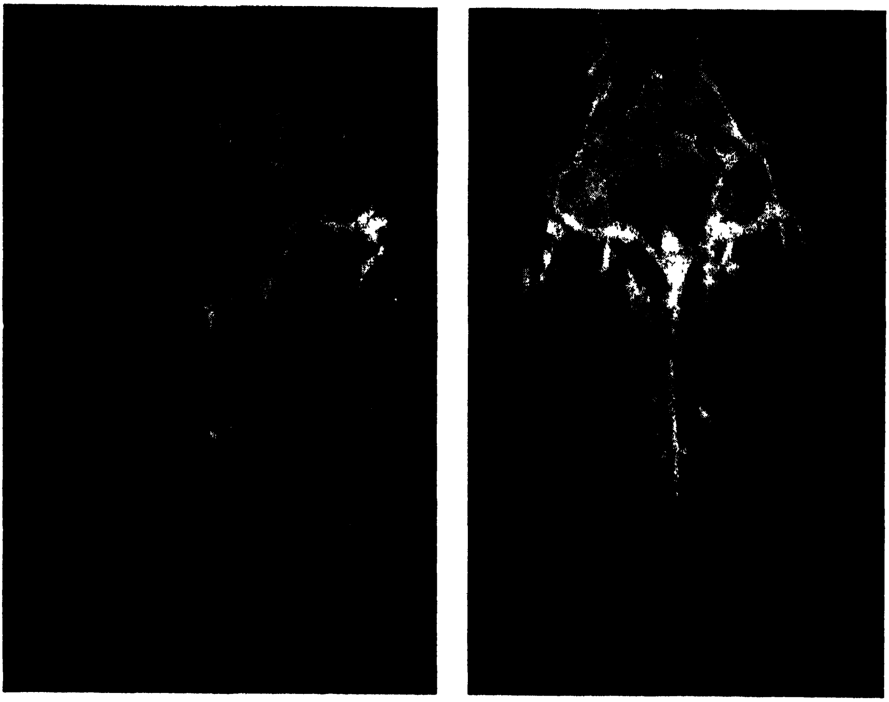

Fig. 144. Cerebral angiogram obtained in a normal rat following the injection of 1 cc. of thorotrast into the common carotid artery immediately after death. Lateral and posterior views. The skull has been decalcified.

(2) On rats in utero, by Job, Leibold, and Fitzmaurice.[4] They found occasional cases of hydrocephalus, and certain abnormalities of the eye and jaw.

(3) On the lumbar sympathetic ganglia of rats, by Griffith and Pendergrass.[5] No demonstrable effect was observed.

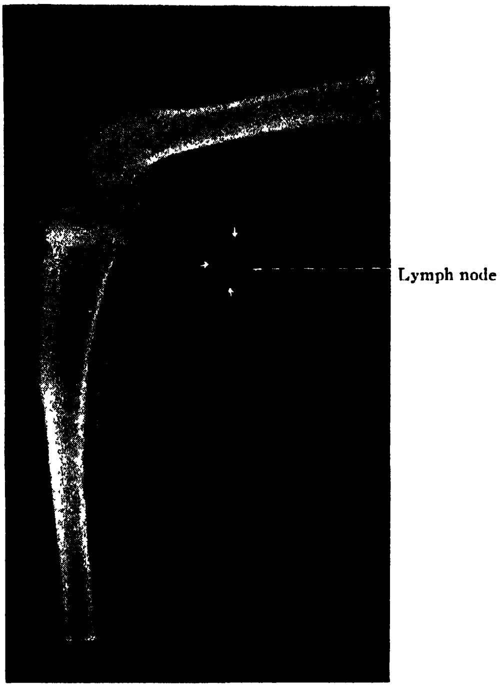

Fig. 145. Roentgenogram of the knee region of a normal rat 30 minutes after the injection of 0.05 cc. of thorotrast subcutaneously into the dorsum of the foot. A lymph node is shown.

X-rays have been used to destroy tissues in certain organs. In the irradiation of the thymus the following technic was employed by Shay and Gershon-Cohen. Rats 48 hours old were used.

(1) The rat is strapped to a board with two narrow strips of adhesive; one across the chest fixing the forelegs and the other across the pelvis fixing the hindlegs.

(2) The rat's chest is then studied fluoroscopically and the center of the thymic shadow is located and its position marked on an adhesive tape placed on the rat's chest.

(3) A lead plate is placed over entire animal. An opening 8 mm. in diameter is made in the lead plate and is placed so that the perforation is centered over ink mark on the adhesive. Lead plate is fixed with thumb tacks to board.

(4) The x-ray therapy is directed to the thymus through this portal, which

is into the superior mediastinum anteriorly. For destruction of the thymus, it was found that it was necessary to give 550 r (measured in air) daily for four

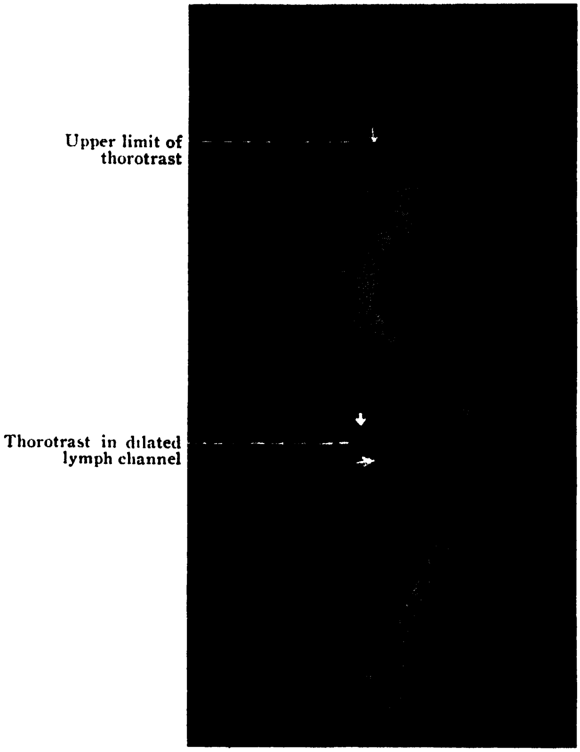
Upper limit of thorotrast
Thorotrast in dilated lymph channel

Fig. 146. Roentgenogram of the leg of a rat with chronic lymphedema. The upper film was made immediately after the subcutaneous injection of 0.05 cc. of thorotrast into the dorsum of the foot, and the lower film was made 30 minutes later. In the lower film the thorotrast demonstrates the dilated lymphatic channels. The lymphedema was produced by an injection of kaolin some weeks later.

successive days. The factors used were 130 KV, 5 M.A., 25 cm. distance; 6 min.; 2 mm. al. filter.

Thymic atrophy was usually complete about ten days after the treatments were instituted.

GASTRO-INTESTINAL EXAMINATION OF RAT

J. Gershon-Cohen and Harry Shay

A water-barium meal is used, 2.5 Gm. of barium being dissolved in 3 cc. of water. The 3 cc. meal is instilled into the stomach of the rat through an 18-gauge needle, 15 cm. long. The tip of the needle is rounded and smoothed. The needle is inserted through the mouth and placed gently in place, the tip

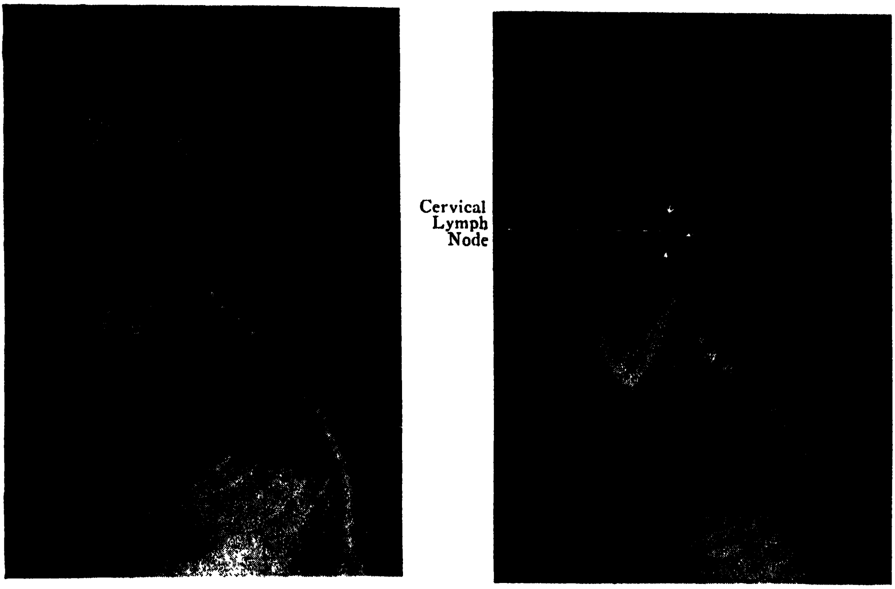

Fig. 147. Roentgenogram of the neck region of a normal rat before and 12 hours after the intracisternal injection of 0.04 cc. of thorotrast. In the latter film a cervical lymph node is shown.

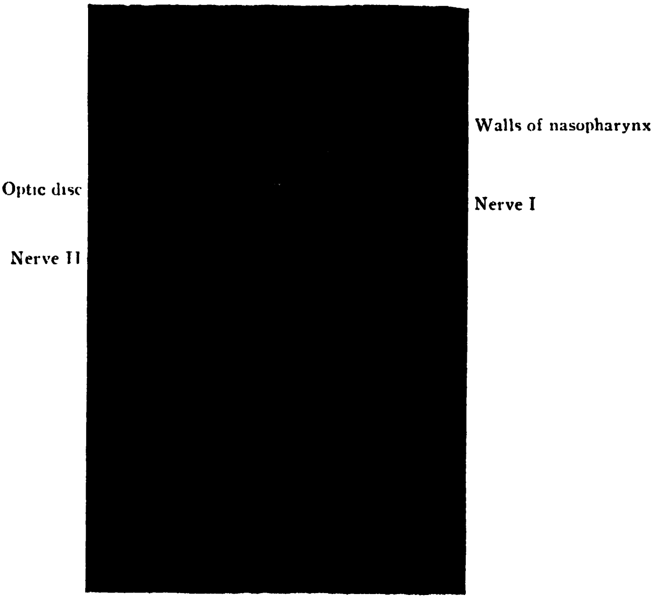

Fig. 148. Roentgenogram of the decalcified skull of a rat following the injection of 0.04 cc. of thorotrast into the cisterna magna.

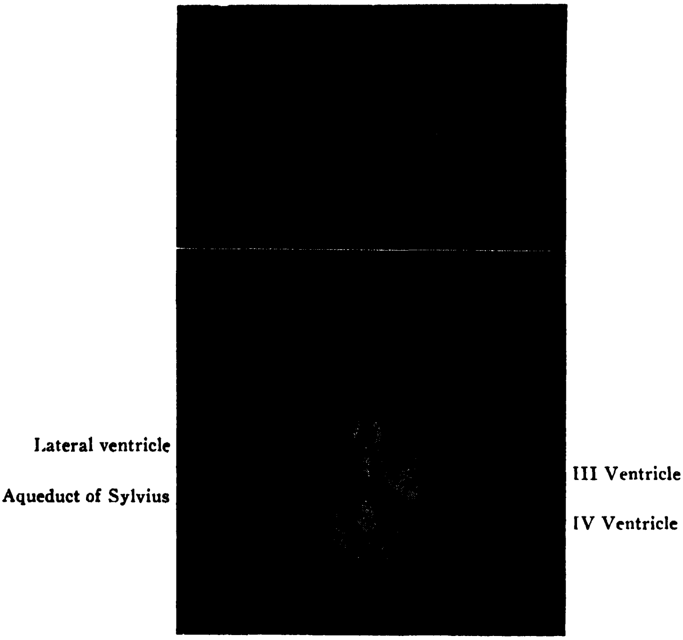

Fig. 149. Encephalogram of a rat after the intracisternal injection of 0.04 cc. of thorotrast in an animal which had previously had an intracisternal injection of a suspension of colloidal kaolin. Note the hydrocephalus.

Antero-posterior and lateral views.

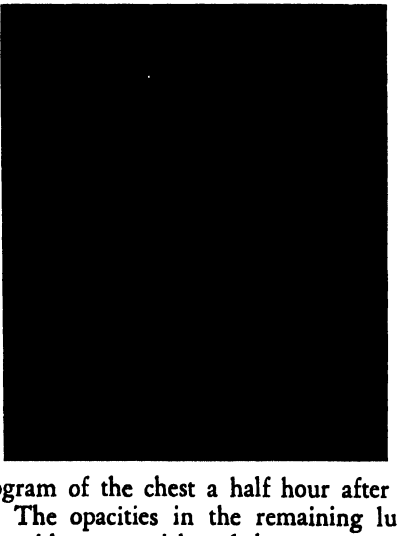

Fig. 150. Roentgenogram of the chest a half hour after the induction of unilateral pneumothorax. The opacities in the remaining lung fields and in the diaphragm on the right side are particles of thorotrast.

Fig. 151

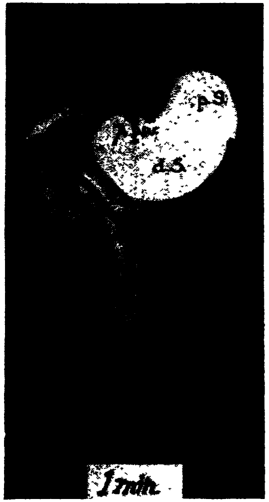

Fig. 152

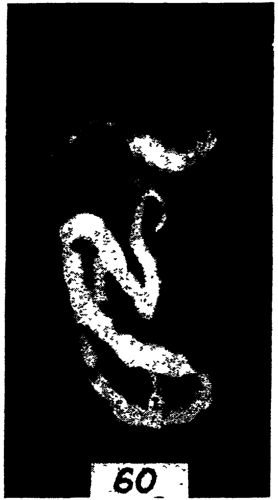

Fig. 153

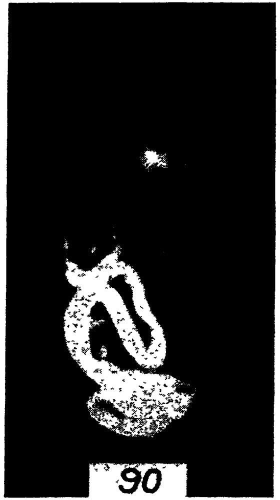

Fig. 154

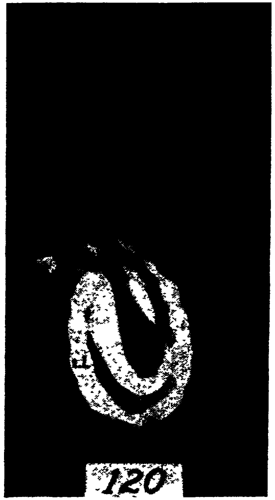

Fig. 155

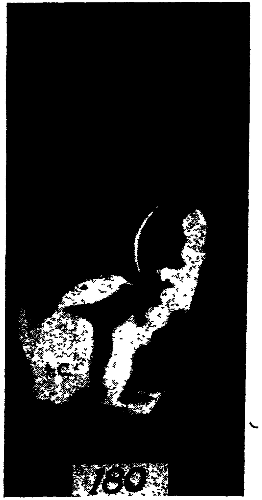

Fig. 151. Roentgenogram of the rat's stomach and duodenum one minute after the administration of the meal. The proximal stomach may be seen capping the cardiac end of the distal stomach. A peristaltic wave traverses the antrum with deeper indentures on the lesser curvature, indicated by an arrow. Time intervals in the following legends refer to time after administration of the meal.

Fig. 152. Sixty minutes. Evacuation from the stomach is practically complete. The longitudinal folds may be seen in the stomach at *A*. The contents are distributed in a continuous column in the lower jejunum and ileum, the margins of which undulate in shallow waves.

Fig. 153. Ninety minutes. The column has now progressed farther into the small intestines where the margins tend to run more parallel.

Fig. 154. One hundred twenty minutes. Some of the ileal loops are now filled by the barium meal.

Fig. 155. One hundred eighty minutes. The column begins to become detached in the terminal ileum and the head of the column has entered the cecum.

Fig. 156

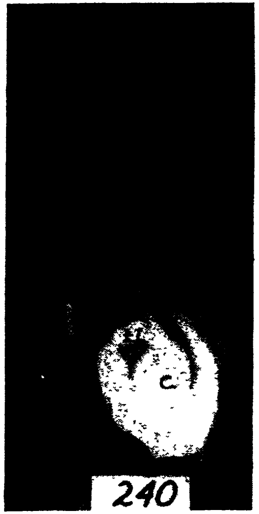

Fig. 157

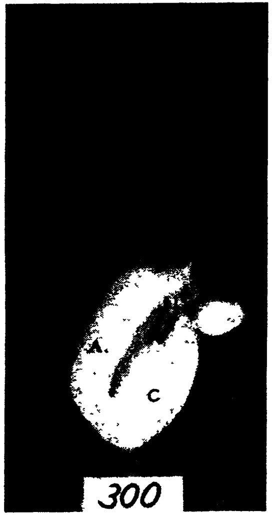

Fig. 158

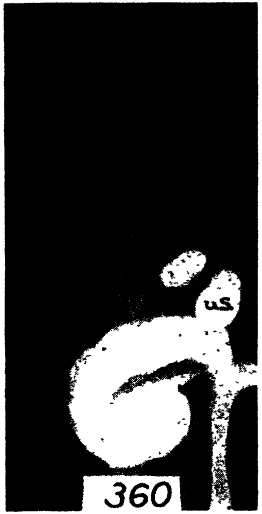

Fig. 159

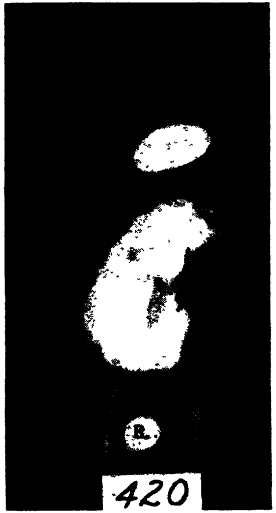

Fig. 160

Fig. 156. Two hundred forty minutes. The cecum is filling with some residue in the terminal ileum.

Fig. 157. Three hundred minutes. The appendix and the cecum are filled, and there is a small residue in the terminal ileum.

Fig. 158. Three hundred sixty minutes. There is no ileal residue at this time and the head of the column in the region of the upper sigmoid (often close to either the hepatic or splenic flexure) has split into small masses ready for evacuation.

Fig. 159. Four hundred twenty minutes. The head of the column has reached the rectum at R.

Fig. 160. Twenty-four hours. The colon is completely evacuated.

coming to rest in the cardiac end about 10-12 cm. from the teeth. An assistant holds the animal by the tail and soft tissues of the back of the neck so as to bring the mouth and esophagus into a straight line. (For other methods of gavage see page 166.)

The same technic of examination is then employed as with the human subject. The meal is given after all food and water have been withdrawn during the previous night, usually 15 hours before the studies begin. The stomach and duodenum are studied fluoroscopically and films are made immediately, at the end of one hour and as often thereafter as necessary to gather the information desired. If the small intestinal tract is to be studied, it has been our custom to make films at least once every hour for 6 hours after injection of the meal. Films are made at the end of each 24 hours until the colon is evacuated if colonic motility is to be studied (Figs. 151-160).

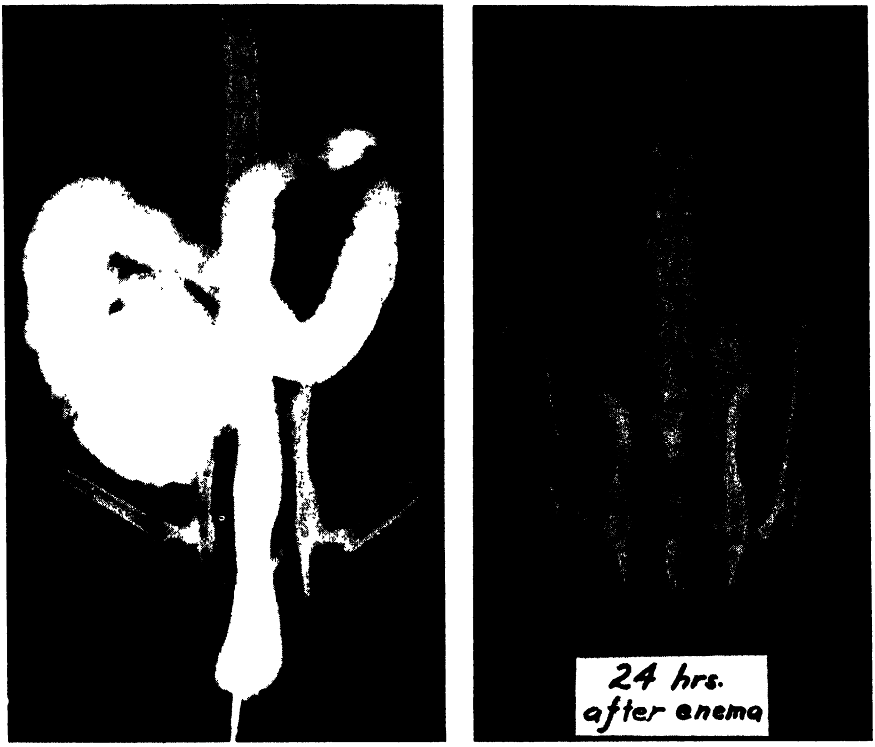

Fig. 161. Barium enema: Films were made immediately after the injection of the barium mixture, and 24 hours later.

A 3 cc. water-barium meal is evacuated from the stomach as a rule in about 2 hours, ranging usually from 1 to 3 hours. The small intestinal tract is traversed by the head of the column in approximately 3 hours, usually ranging from 2 to 4 hours. The small intestines are usually empty in 9 hours, ranging from 6 to 12 hours. The emptying time of the colon is approximately 24 hours; rarely ever is a residue seen beyond 48 hours.

A 3 cc. water-barium meal usually fills the stomach. Its division into the cardiac and distal portions can be demonstrated nicely with a meal of this size. Peristaltic waves are seen usually only in the distal stomach, very much as in the human subject. A trial portion of the opaque contents often rushes through the pylorus immediately upon filling the stomach, but intermittent delay in emptying may be observed. The cap is not usually visualized separately from the rest of the duodenum and in order to demonstrate what corresponds to the cap, rotation of the animal is usually necessary. Rugal markings and the markings of the valvulae conniventes are not seen as in the human. The duodenum is usually about 25 mm. in length; its exact length is difficult to measure. The jejunum and ileum fill and empty very much as in the human subject, but the column is usually more continuous and the flecking and striation caused by mucosal markings of the jejunum and ileum, as seen in the human, are not seen in the rat. The margins of the small intestinal tract tend to be more or less parallel, excepting where peristaltic waves are in progress. These waves are shallow and relatively long, resulting in an undulating-like contour. The jejunum and ileum together usually measure from 80 to 100 cm. in length and are cleared by a 3 cc. water-barium meal, usually between 6 and 9 hours.

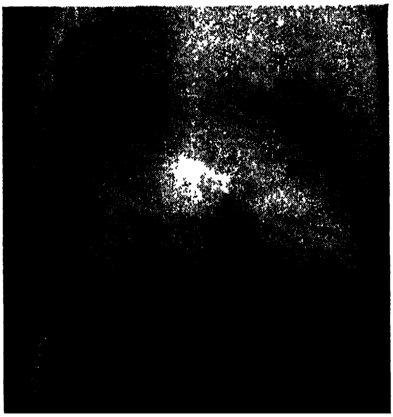

Fig. 162. Roentgenogram showing the shadows of the liver and spleen produced by thorotrast. The rat received 1 cc. of thorotrast intravenously daily for 3 days, and the picture was taken on the 5th day.

The appendix really appears like part of the cecum and is often so poorly defined as not to be distinguishable from the cecum; usually however, there is a slight constriction which divides the cecum into two parts, an apical and a basal portion. The apical portion constitutes the appendix; and as in the human it varies greatly in length—usually from 10-30 mm.

The head of the column of opaque contents usually enters the colon between the second and fourth hour. The colon varies from 15 to 25 cm. in length. The opaque contents usually fill the right side in a uniform, continuous column and resemble a large, sausage-shaped mass while the left-side column is broken up into separate detached masses shaped like olive-stones, characteristic of the feces as they are evacuated. There are usually several colonic evacuations during the twenty-four hour interval, at the end of which time most of the opaque contents are evacuated.

A barium enema also may be given to the rat, either with or without the use of narcotics, sedatives, or other drugs. We have found the use of sodium Amytal very effective, using from 0.4-0.6 cc. of a 3% solution in rats 200-300 Gm. in weight. The cannula that serves for direct gastric instillation may be used for injecting the colon. After the tip has been passed into the rectum about 8 mm., a ligature of ordinary cotton string is passed around the tail and skin just proximal to the anus and tied securely in place. This prevents regurgitation. The colon is then filled either by gravity flow or by the use of a plunger-syringe under fluoroscopic control. Films may be made using the same technic as employed with the mouth meal studies. Sparks and Collins,[6] whose method of using the enema in the rat has served as our guide, have found the capacity of the average colon to range from 3-7 cc. without the use of soporific drugs while we found ranges from 6-15 cc. when sodium Amytal was used. One encounters the same difficulties in achieving uniform filling of the colon as in the human. There is an easy regurgitation of the contents through the ileo-cecal valve, but reduplication and redundancy are not encountered to interfere with adequate demonstration of the entire length of colonic margins.

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16

Surgery of the Rat

By

DWIGHT J. INGLE and JOHN Q. GRIFFITH
Assisted by W. A. Jeffers, M. A. Lindauer,
H. U. Hopkins and Albert Segaloff

GENERAL PRINCIPLES

RATS are less susceptible to postoperative infection than are many other animals. As a matter of fact, if all aseptic precautions are disregarded, but the operation is carefully performed and the animal kept in a dry cage, many of the animals will remain free from infection after most procedures. Occasionally infection occurs leading to abscess and breaking down of the wound. After abdominal incisions such infection may be followed by herniation. Although complete asepsis is desirable, expediency may justify less rigid technic for some operations. Some important precautions we have adopted are: (1) Do not leave blood clots; they usually become infected. (2) Do not sew hair into the edges of the wound. The hair must be clipped and shaved before the operation. (3) Approximate fascial or skin edges with interrupted stitches about an ⅛ inch apart. Continuous sutures cause puckering and, sometimes, necrosis. (4) Use cutting edge needles only for the skin, round needles elsewhere. In very young rats round needles should also be used for closing the skin. (5) Remove sutures from the skin on the 7th day. (6) Keep the cages dry for at least 24 hours postoperatively. We use newspaper, changed frequently.

Our operative procedure is as follows: (1) The operative board is of soft wood approximately 8 x 10 inches in size. The board is provided with hooks placed at distances of one inch apart around its edges. It is covered with a clean towel. The rat under anesthesia is secured to the board by means of rubber bands looped about the feet and over the hooks. (2) The operative area is clipped with scissors and then shaved. (3) It is painted with a skin disinfectant. We have used 95% alcohol, or 5% tincture of iodine, or tincture of Metaphen (Abbott). All are satisfactory but we prefer the last. (4) The tray containing the operating instruments, thread, needle, and gauze, is removed from the sterilizer and permitted to cool. (5) The hands are scrubbed with soap and water, using a brush and are dried with a clean paper towel. If the animal is more valuable, the hands are rinsed in alcohol and allowed to dry in air. (6) The operation is performed. In general, we prefer scissors to knives. Hemostats are seldom necessary. Only the principal veins and arteries, if injured, require ligation. (7) After the operation, the fascia and skin are sutured as described above with fine linen or silk thread. Catgut may be used for buried sutures, but it is expensive and not necessary. The ends of buried knots are cut short, while those of skin sutures are left long to facilitate removal. Bandages and dressings are never used. (8) The rat is wrapped in a towel and kept in a warm place until out of anesthesia. It is then returned to a dry cage. If the anesthetic is ether, recovery may be complete in a few minutes and the towel may not be necessary. Unconscious rats must never be returned to a cage containing other rats. It is advisable to keep freshly-operated rats from the company of other animals until the wounds become completely dry. (9) Seven days later the dermal sutures are removed. If these are few, the rat may simply be held by an assistant; or a short ether anesthesia may be employed.

When an operative technic is found difficult to master, it is advisable for the experimenter to practice each step of the operation separately upon dead rats.

Standard Operating Set.

Two pairs of scissors, one medium size and one small.

Three pairs of small forceps without teeth, two with curved tips and one straight.

Two pairs of tooth forceps, small, one with curved tips and one straight.

Two pairs of small artery forceps.

One cutting edge needle, medium size, straight, and one straight round needle.

Size No. 0 linen or No. 2 silk surgical thread, wrapped around a short glass rod.

HYPOPHYSECTOMY

The following procedure is a modification of the method first described by Smith.[1] Ether anesthesia is preferable. The animal is immobilized on its back by means of rubber bands looped about the legs. The head and neck are kept extended by a rubber band looped over the upper incisors. A rubber tube is inserted into the mouth in a manner which depresses the tongue and permits the animal to breathe through the mouth. The neck is shaved and then painted with tincture of Metaphen. The operation is performed with the head of the animal toward the operator. Illumination is provided by a head-lamp or by a small spot-light. A midline skin incision is made extending from the mandibular papilla to the lower border of the submaxillary gland. The salivary glands and subcutaneous tissue lying to the right are freed by blunt dissection and retracted to the right by means of a small hemostat. Longitudinally at the lower border of the larynx and laterally at the border of the trachea the closed tips of fine, smooth, curved forceps are forced through the omohyoid muscle at the point of its insertion under the sternohyoid muscle. The tips of the forceps are directed through the muscle toward the midline (Fig. 163) and are then spread apart to make a longitudinal division of the muscle extending from the point of its upper attachment to the level of the lower border of the thyroid. Spreading the forceps laterally and retracting toward the head will expose the area to be prepared for drilling. (Fig. 164.) This step of the operation should be carried out without producing hemorrhage and should give a clear view of the area to be prepared for drilling without additional dissection and without damage to the nerves and vessels of this region. (The trachea is almost completely closed and therefore this retraction can be maintained for only a few seconds at a time. When respiratory movements cease they can be readily restored by promptly inserting a rubber tube into the mouth of the rat and blowing air directly into the lungs.) The bony ridges representing the occipito-sphenoid synchondrosis and the crista occipitalis are exposed by scraping away the overlying muscle and finally wiping the area dry with cotton pellets twisted on toothpicks as applicators. The pharynx must not be entered. The pituitary is exposed by means of a long shank, round bone-burr attached to a dental drill. Either a foot engine or an electric motor controlled by a foot operated rheostat may be used to drive the drill. The size of the burr required is determined by the size of the rat. A No. 9 dental bone-burr is satisfactory for operations on rats weighing 150-200 grams. The hole is drilled in the midline on the occipito-sphenoid synchondrosis (Fig. 165) in a manner which allows approximately two-thirds of the hole to lie cephalad of the suture line. The drill is not allowed to break through the bone, but is carefully controlled so that the last shell of bone is drilled away, preferably without rupture of the

Fig. 163

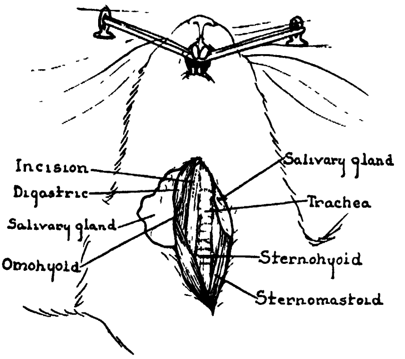

Fig. 164

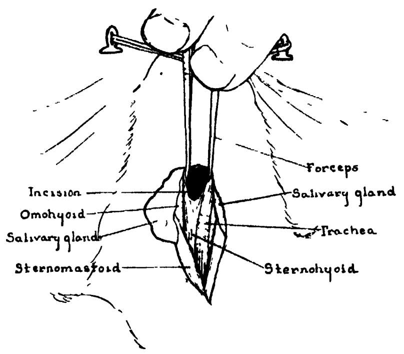

Fig. 163. Anatomical structures of the neck exposed in the first stage of hypophysectomy. The dotted line indicates the site and approximate length of the incision through the omohyoid muscles.

Fig. 164. Stage following Fig. 163. The lateral spreading of the tips of the forceps and retraction toward the head exposes the bony ridges of the occipitosphenoid synchondrosis and the crista occipitalis.

membranes covering the gland. A pick suitable for manipulation of the membranes and of the pituitary can be prepared by breaking the shaft of a 22 gauge needle from its base and fixing it in a slender, removable handle. For most purposes the sharp point of the needle should be converted into a fine hook by blunting. The pituitary is exposed by tearing away the dura. The operative field is dried when necessary by the use of cotton pellets. A pipette of the largest size accommodated by the drill hole is placed against the pituitary and the gland is removed by negative pressure. The strength of suction is regulated by a foot-control. The suction should not be stronger than is necessary to draw the gland into the pipette. The pipette is attached by means of a two-hole rubber stopper to a glass tube having the approximate dimensions of ¾ inch diameter and 4 inches length. The tube acts as a suction trap and allows the tissue to be visualized and examined after removal. When properly removed, the anterior lobe is usually broken into two parts and the posterior lobe is separated from the anterior lobe but is unbroken.

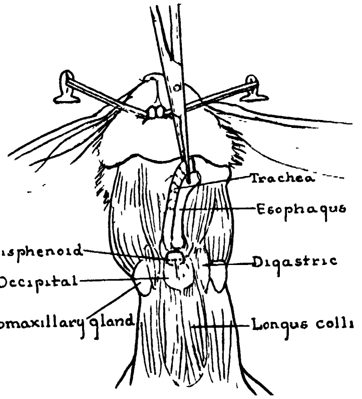

Fig. 165. An extended dissection of the neck region (not a stage of the operation) in which the dotted line indicates where the drill hole should be placed.

In order to remove the anterior lobe of the pituitary while leaving the posterior lobe intact the drill-hole is placed slightly forward so that the cephalad border of the anterior lobe is exposed. The anterior lobe is then divided through its midline with a sharp hypodermic needle. A pipette of not more than one millimeter diameter at its tip is placed against a half of the anterior lobe as far to the left or to the right as possible. This portion of the gland is removed by gentle suction. The remaining portion of the anterior lobe is carefully manipulated to the center of the drill-hole with a fine, hook-tipped needle. It is then removed by suction. The posterior lobe can be left intact in a high percentage of cases by this procedure.

For the removal of the posterior lobe the bone-burr used should be approximately one-half the diameter of the burr required for total hypophysectomy. The cephalad border of the anterior lobe is exposed. A fine pipette is made to penetrate the anterior lobe tissue and to come in contact with the posterior lobe before suction is applied. It is then possible by means of a careful control of the suction to hold the posterior lobe against the tip of the pipette and to remove it without drawing it completely into the tip. In our experience this operation is likely to result in failure in at least 50% of the cases. Others who are more skilled in experimental surgery may attain a higher degree of success.

Following the removal of the pituitary tissue the exposed tissues are wiped free of blood and the skin incision is closed by suture. The animals should be kept in dry cages in a warm room. A temperature of 28°C. is optimal. The completely hypophysectomized rat is prone to develop hypoglycemia and may succumb. This deficiency can be compensated for by adding sucrose in an amount of 5% by weight to the drinking water.

In order to determine whether the posterior lobe of the pituitary has been removed without destruction of the anterior lobe, there must be a careful histologic examination of serial sections made of the tissues remaining in that area. As a criterion of an anterior lobe deficiency resulting from hypophysectomy the loss of body weight, and the regression of the thyroid, adrenal cortices, and gonads provide a satisfactory index. However, if it is important to establish whether or not the removal of the anterior lobe was complete, this must be determined by serial section and histologic examination of the tissues remaining in that area.

TRANSPLANTATION OF THE ANTERIOR LOBE OF THE PITUITARY

Viable grafts of anterior lobe tissue may be obtained but no method has been established whereby such grafts can be made to maintain a normal level of functional activity. A number of experimenters have now obtained viable autoplastic and homoplastic grafts by transplanting the tissue into the anterior chamber of the eye by the method of Markee.[2]

PINEALECTOMY

The following description of technic is taken directly from a paper by Anderson and Wolf.[3] Rats 1-3 days old are used. Aseptic precautions are observed. A dorsal midline incision is made in the skin of the head. A square skull flap is raised by incising with a small lancet at a point over the convexity of the right lobe of the cerebellum, and extending the incision to the left about 1 mm. past the midline. The incision is continued anteriorly from both the right and left ends to a point about 3 mm. anterior to the transverse sinus. The dura is then separated from the skull and the square flap of skull lifted up and bent anteriorly. The dura is incised over the cerebrum just to the right of the pineal. The pineal is thus exposed and is now removed with forceps. Hemorrhage is controlled by light pressure with cotton. The bone flap is replaced and the skin sutured.

THE IMPLANTATION OF BRAIN TUMORS

By W. A. Jeffers

Rat sarcoma R39 (obtained through the courtesy of Dr. F. C. Wood, Columbia University, New York) can be readily transplanted from the donor rat and is kept warm and moist with gauze sponges wrung from warm, sterile, physiologic salt solution. Recipient rats* must be tumor susceptible for best results. After anesthetizing the animal with ether, the skull is exposed, and a small hole is drilled through the bone. A small bit of fresh tumor tissue is drawn up in a micro-pipette of glass and is expelled into the brain substance. The periosteum and skin are carefully sutured over the trephine hole. A tablet of mercurophen, dissolved in 100 cc. of a 0.85% sodium chloride solution, furnishes a satisfactory antiseptic. Alcoholic antiseptics are not used, as they may kill the tumor implant. Signs of tumor growth are usually apparent within ten days.

METHODS FOR MAINTAINING A DECAPITATE PREPARATION

By M. A. Lindauer

The respirator used was designed by Mr. Arthur Rawson. It is illustrated in Figure 166 and the principles of its manufacture described in the accompanying legend by Mr. Rawson. The compressed air outlet may be connected through a T-tube with the motor inlet and the inlet for the air supplied to the animal, as shown in Figure 166. It is usually desirable that the air supplied to the animal be less than that passing to the motor. This can be arranged by placing a screw clamp upon the rubber tube going to the animal inlet. The rubber tube passing to the animal goes to a glass T-tube, one end of which is drawn out to fit loosely into the trachea of the animal. The open end of the glass T-tube is fastened to a short length of rubber tubing which is encircled by a screw clamp. By adjusting these clamps the volume of air reaching the animal may be regulated.

The rat is first anesthetized by ether and a ventral incision is made in the neck. The trachea and main vessels are dissected free. A tracheal cannula attached to the respirator is inserted and adjusted. The vessels are then tied and the animal decapitated above the level of the ligature. This gives a preparation from which all central nervous system control has been destroyed. Our work has not required the maintenance of such a preparation for more than an hour, though it has usually been in good condition at the end of this period.

THYRO-PARATHYROIDECTOMY

The head of the animal is kept extended by means of a rubber band which is looped over the upper incisors. A ventral midline skin incision is made extending slightly beyond the upper and lower borders of the submaxillary gland. The salivary glands are freed at the midline by blunt dissection and retracted laterally. The sternohyoid muscle is divided in the midline from the hyoid through about one-half the distance to the manubrium. Smooth, curved forceps with the closed tips pointing upward and directed toward the head are inserted through the incision at the level of the thyroid and are then permitted to spread apart, thus exposing the thyroid by retracting the muscle. (Fig. 167.) The isthmus of the thyroid is divided in the midline. The body of the thyroid lobe is then clamped tightly by means of fine curved forceps containing teeth in the tips. The gland is gently pulled upward and laterally and is dissected free from its attachments by means of a sharp needle. The recurrent laryngeal nerve must be visualized and carefully avoided during

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* Such animals can be obtained from the Rockland Farms, New City, N. Y.

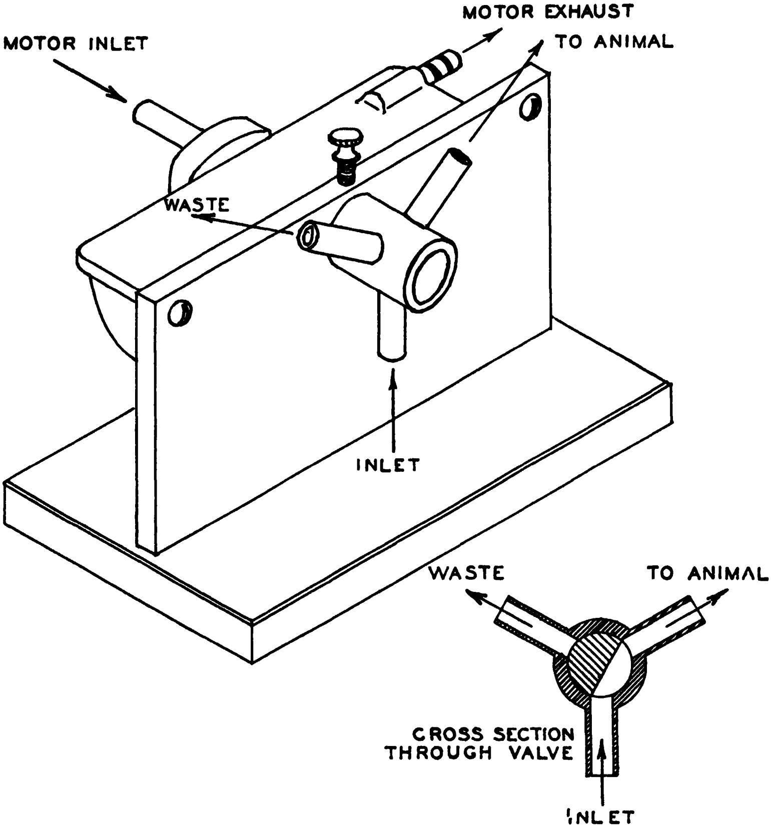

Fig. 166. Air-driven Respirator

The motor is made from a standard pneumatic windshield wiper by fitting an inlet to the valve case and sealing the latter airtight to the body of the wiper The original vacuum connection of the wiper then serves as an exhaust The valve consists of a body with three ports, within which a notched plug oscillates through an angle of approximately 120° so as to connect the inlet port first with one and then the other of the two remaining ports The plug is keyed to the motor shaft The valve body can be rotated through a small angle and locked in any desired position by means of a knurled screw In this way the length of time during which air is supplied to the animal can be adjusted The function of the waste port is to reduce the fluctuations of pressure in the air supply line The motor speed and hence the respiration rate can be adjusted by controlling the air supply to the motor.

the dissection. Hemorrhage is slight if the thyroid artery is kept under tension and severed by picking with a needle. The objective of the operation should be to remove, intact, each lobe of the thyroid without injury to the recurrent nerve. The edges of the muscle are pulled together but are not sutured. The skin incision is closed with silk. During the operation, clean but not necessarily aseptic technic should be employed. The rat does not readily become infected in the region of the neck.

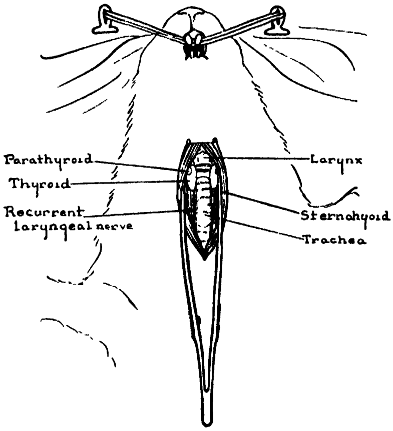

Fig. 167. Anatomical structures of the neck involved in thyroidectomy. Forceps are inserted through the incision, the tips penetrating the divided portions of the sternohyoid muscle. The blades of the forceps are permitted to spring apart thus displacing the divided portions of the muscle laterally and exposing the thyroid.

The very young rat is prone to develop parathyroid tetany following this operation. This may be prevented by the administration of calcium salts or by the injection of parathyroid extracts for a few days postoperatively. Following recovery from the immediate effects of the operation, tetany is not likely to occur. When the operation is employed to produce a state of thyroid deficiency its success must be judged on the basis of some outside criterion, such as depression in the rate of general metabolism. In the case of very young rats the retardation of body-growth, loss of hair, dryness of the skin, inactivity, etc., may be accepted as criteria of thyroid deficiency.

CAROTID SINUS DENERVATION

The carotid sinus nerve is not visible macroscopically in the rat. A sharp rise in blood pressure follows section of this nerve. Therefore the animal is arranged for blood pressure measurement (Fig. 129, p. 276) and a reading is obtained before starting the operation. The common carotid artery is exposed by a longitudinal lateral-neck incision. Care is taken to avoid injury to the vagus nerve. The dissection is carried upward to the point of bifurcation of the common carotid to form the internal and external carotids. The fork especially is carefully dissected free. A second blood pressure reading is then taken. If a sharp rise has occurred, usually to 180-200 mm., it is assumed that the nerve has been cut. If the blood pressure is unchanged, the dissection is continued. If bilateral operations are planned, it is advisable to operate on the other side several days later. The blood pressure will have returned to normal by that time and a rise may again be used as a criterion of successful nerve section. Moreover, the two-stage operation has a lower mortality.

PNEUMOTHORAX

By H. U. Hopkins

The technic of inducing pneumothorax in the rat is similar to that used in man, with due allowance for the difference in thoracic volume. A simple apparatus has been assembled which permits measurement of the volume of air injected and observations on the intra-pleural pressure. (Fig. 168.)

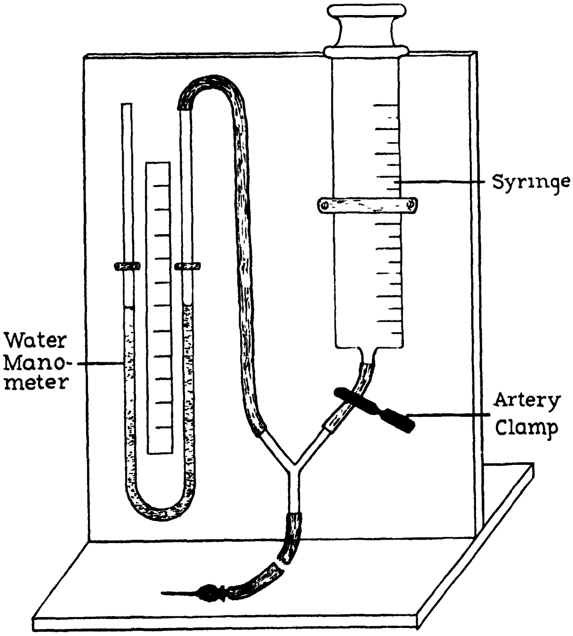

Fig. 168. Diagram of apparatus used in inducing pneumothorax. For description of operation see text.

The manometer is formed from small-bore glass tubing. A 5 cc. syringe is used. The rubber tubing is of the standard type used for blood pipettes. A small bore (No. 22 or 24), short-bevel needle is most satisfactory.

Technic. The artery clamp is released and the plunger of the syringe is drawn upward; the clamp is then closed. If the plunger tends to fall, due to imperfect fit in the barrel, a film of light oil will effectually seal it. The needle, connected by the tubing with the manometer, is then slowly inserted perpendicularly through the skin and tissues. The site of the puncture is not of great importance; the lower halt of the thorax is usually preferred. When the needle passes through the parietal pleura, the water manometer will show a definite negative pressure which fluctuates with respiration. The clamp is then released. The weight of the plunger is usually sufficient to force the air into the pleural cavity. It is preferable to control the plunger with one hand to prevent too rapid a flow of air. The filling should be done slowly, with constant observation of the manometer to avoid a positive pressure. Too rapid filling or a positive pressure may rupture the delicate mediastinal structures. When the manometric pressure approaches zero and the respiratory fluctuations have practically disappeared the needle is withdrawn. The small puncture wound closes at once, without loss of air.

THYMECTOMY

By Albert Segaloff

The thymus is most readily removed from rats of 35-40 grams. Deep ether anesthesia is best, as the animal usually shows a short period of postoperative respiratory difficulty and, consequently, the longer anesthesia induced by the barbiturates should be avoided. If the anesthesia is not deep enough the animal will struggle and the lungs may be extruded through the incision and injured seriously.

The animal is placed on its back with the head toward the operator. A midline incision is made through the skin and superficial fascia from the level of the angle of the mandible to the level of the sixth rib. A small incision is then made in the deep fascia, and the salivary glands and sternohyoid musculature are then separated in the midline, exposing the trachea down to the manubrium sterni.

At this stage, in order that the subsequent thoracic closure be made easier, it is best to tie the suture for thoracic closure into the pectoralis muscles at the level of the fourth rib.

A longitudinal incision is then made with iridectomy scissors through the manubrium and the sternum slightly beyond the level of the third rib; care must be taken to keep the point of the scissors against the dorsal aspect of the sternum. The thymus can be seen through this incision.

After the cervical extensions of the thymus are freed by blunt dissection the gland is grasped firmly in a moderately-blunt curved forceps. While firm traction is being applied to the gland through the forceps, a cotton-tipped toothpick is used to free the thymus gently from the surrounding structures. Too much traction on the gland, or carelessly freeing it from the underlying tissue may lead to rupture of the underlying great vessels and subsequent loss of the animal. The thymus frequently separates into two lobes which must be removed separately. If this stage is carefully performed there is little or no bleeding. Any hemorrhage, short of rupture of the great vessels, can be controlled by cotton applicators. After removal of the gland the sternum is approximated by a continuous suture placed in the pectoralis muscles and the skin closed as usual.

The mortality from this operation is about 5%. The animals usually start to gain weight on the day following the operation. In older animals greater care must be used in removing the gland, as the surrounding connective tissue is more dense than in the younger animals.

ADRENALECTOMY

Ether is the anesthetic of choice, but one of the short-acting barbiturates may be used. A single dorsal midline incision is made through the skin at the level of the kidneys. After retraction of the skin to the right or left, the muscle is exposed by cutting through the subcutaneous fascia. A longitudinal incision is made along the lateral border of the dorsal muscle mass, from the level of the center of the kidney toward the apex of the angle made by the lowest rib and the dorsal muscle mass. The small blood vessels of this region must be avoided. The adrenal is located at the upper pole of the kidney. The fat tissue lying between the adrenal and the kidney is held with fine-toothed curved forceps and pulled into the incision so that the adrenal is brought into view. The gland together with its adherent mass of fat is freed from the upper pole of the kidney and from the overlying muscle so that the only attachments are through the pedicle of the adrenal. The pedicle is clamped with small, tightly gripping artery forceps at a safe distance from the adrenal, and fine, smooth-tipped curved forceps grip the pedicle directly under the gland and pull gently upward so that the area of the pedicle between the two pair of forceps is slightly stretched. The gland is then freed by cutting the pedicle close to the artery forceps. The forceps are left tightly clamped on the central stump of the pedicle for one or two minutes to insure hemostasis. The second adrenal is then removed. The primary objective of the operation is to remove the adrenal with its adherent fat tissue without rupturing the capsule of the gland. It is important to avoid any unnecessary trauma or loss of blood.

The period of survival of untreated adrenalectomized animals is influenced by postoperative care. The animals are kept in clean, dry cages. The optimal temperature for survival is approximately 28°C. The balance of electrolytes in the diet is an important factor. Long periods of survival are favored by diets which are high in the content of sodium and chloride and low in the content of potassium.

ADRENAL DEMEDULLATION

The most successful method for removal of the adrenal medulla in the rat is the enucleation technic first described by Evans.[4] Following exposure of the adrenal body, the pedicle is lightly clamped below the gland so that its vessels are not crushed. The distal tip of the gland is clipped away with small, sharp scissors. Fine, smooth-tipped, curved forceps are inserted about the base of the gland and pressure is gently applied to the sides and progressively toward the open capsule. The body of the gland is easily popped out of the capsule in this manner leaving the capsule, together with an adhering layer of glomerulosa cells, attached to the pedicle. A mass of cortical tissue regenerates from the capsule and its glomerulosa layer, which is usually adequate to meet the physiologic requirements for the cortical hormone. During the first week following adrenal enucleation the animals are maintained on a high-sodium chloride, low-potassium diet with an additional 1% solution of sodium chloride to drink. Three to four weeks are usually required for the complete regeneration of the adrenal cortices. Tiny islands of chromaffin tissue may infrequently be found in the regenerated cortical tissue, but they are never of sufficient amount to be of functional signficance.

ADRENAL TRANSPLANTATION

Autoplastic grafts of adrenal glands in the rat can be made with uniform success. Homoplastic grafts are successful only when there is a close genetic relationship between the host and donor.[5] We have not been able to make successful grafts of adrenal tissue from other species of animals to the rat. Growth of transplanted adrenal tissue does not occur in the presence of other functioning adrenal tissue although it is possible for the graft to remain viable under such conditions. The recipient should be completely adrenalectomized at the time the graft is made. Under the usual conditions only the cortex of the adrenal survives and regenerates, although chromaffin tissue may survive if it is placed directly in contact with the host tissue. Strict aseptic precautions must be observed in these operations.

The best method for securing successful grafts of the adrenal cortex is to transplant the whole adrenal to the surface of the ovary of the sexually-mature female rat. The whole gland is removed from the donor, carefully cleared of its surrounding fat, and attached to the surface of the ovary by means of a fine strand of silk which passes through the body of the adrenal and around the body of the ovary. Care must be taken to prevent hemorrhage at the ovary since the presence of clotted blood hinders the establishment of vascular continuity between the graft and the host tissue.

The adrenal glands of the rat can be transplanted to other parts of the body. We have had good success with transplants of the adrenals to the groin. The femoral vessels of the groin are exposed and visualized between the level of the superficial epigastric and muscular branches and the point at which the femoral vessels enter the popliteal space. By means of a smooth-tipped probe a small opening is made in the subcutaneous fascia and a small pocket is formed under the femoral vessels, so that the adrenal body can be gently forced into the pocket and made to lie under the femoral artery, just central to its epigastric branches. The opening in the fascia is closed with silk. A second adrenal gland may be transplanted into the groin of the opposite leg.

Following operation these animals should be maintained for one week on a low-potassium, high-sodium chloride intake with the addition, if necessary, of minimal maintenance doses of cortin. Successful grafts become functionally active within a few days following operation so that special care is no longer necessary. The regeneration of cortical tissue within the graft should be completed within four weeks following operation.

NEPHRECTOMY

An incision is made in the flank, extending from the costal border to the iliac crests. The kidney may be delivered by aid of a finger inserted into the wound or by pressure at the sides of the wound. The adrenal is displaced upward and detached from the upper pole. The pedicle of the kidney is ligated with a single ligature and the organ is excised. If it is desired to remove only a part of the organ, ligatures may be pulled through its substance and tied so as to eliminate functionally the upper and lower thirds.

SPLENECTOMY

A longitudinal incision is made on the left side of the abdomen extending about an inch below the costal border. The spleen is delivered through the wound, the pedicle is ligated with a single ligature, and the organ is excised. In rats which are infected with Bartonella Muris the removal of the spleen is followed by development of a marked macrocytic, hyperchromatic anemia which usually results in the death of the animal.[6] Bartonella-free rats can be expected to remain in good health following splenectomy.

PARTIAL PANCREATECTOMY

This is a modification of the technic described by Shapiro and Pincus.[7] The operation can be performed most successfully on rats weighing from 45 to 75 grams. A ventral midline incision is extended caudal-ward from the tip of the xiphoid process for the shortest distance which will permit visualization of the entire duodenal loop. The animal is rotated so that its head points to the left of the operator. In this position the duodenum lies slightly under and anterior to the transverse colon. The duodenum is pulled through the wound and stretched over the colon and away from the operator so that the entire duodenal loop is exposed. (Fig. 169.) The exposed intestines and the fingers of the operator are kept moist with 0.85% sterile saline. Using fine, smooth, curved-tipped forceps the pancreas is picked away from the mesentery of the duodenal loop. The blood supply to the gut must be preserved and the mesentery must not be destroyed. Care must be taken to avoid damage to the bile duct, therefore the pancreatic tissue lying directly over it must not be disturbed. When all of the available pancreas has been removed from this side of the mesentery the duodenum is then retracted toward the operator, and the stomach, spleen, and transverse colon are extruded. A great part of the remaining pancreas is distributed throughout the greater omentum (Fig. 170) which lies as a collapsed tent with attachments to the spleen, pyloric end of the stomach, duodenum, and transverse colon.

The pancreatic tissue is removed from the spleen by placing the index finger of the left hand under the omentum and rubbing free with the closed tips of smooth forceps the part lying distal to the splenic vessels. In a similar manner it is removed from the pyloric end of the stomach. The remaining bits of pancreas are picked away from the splenic vessels. Additional tissue is now removed from the duodenal loop as its mesentery is exposed by retraction of the duodenum toward the operator. All of the remaining tissue is to be removed except that lying between the bile duct, the pyloric end of the stomach and the duodenum. It is important that some acinar tissue remain if it is desired that the animal maintain a good state of health.

Fig. 169

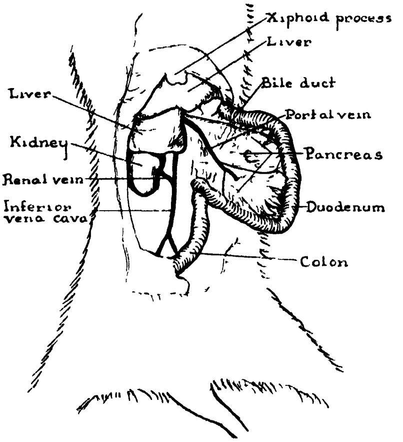

Fig. 170

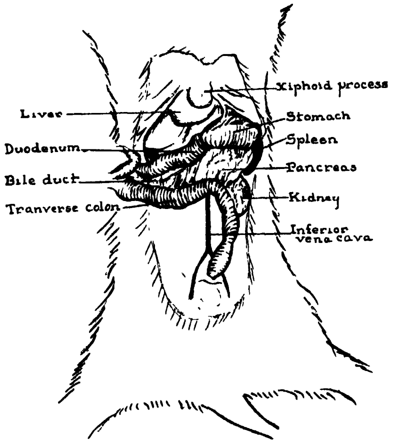

Fig. 169. An extended dissection (not a stage of the operation) showing that portion of the pancreas which lies within the duodenal loop.

Fig. 170. An extended dissection (not a stage of the operation) showing the greater omentum and its pancreatic tissue lying between the duodenum and the transverse colon.

At the completion of the operation the organs are replaced in their normal positions. The animals must be kept warm following operation. We routinely give partially depancreatized rats physiologic saline to drink for five days following operation.

The errors which lead to an unsuccessful operation are: loss of blood to the point of producing a state of shock; damage to the bile duct, which causes obstructive jaundice; trauma to the stomach or duodenum; destruction of their blood supply and supporting structures, which lead to intestinal obstruction; and incomplete removal of the pancreas. Our operative mortality for this operation is about 10%. Approximately 50% of our animals show a spontaneous glycosuria when fed, while all of them are more sensitive to diabetogenic agents than are normal rats. Glycosuria usually does not appear in partially depancreatized rats until the period of rapid body-growth is passed.

HYSTERECTOMY

A midline ventral incision is made extending from the level of the urinary bladder to the level of the lower poles of the kidneys. The uterus is ligated at the cervix and between the oviduct and ovary of each side. The cornua are then excised by section through each oviduct and through the cervix.

OOPHORECTOMY

A single longitudinal skin incision is made in the dorsal midline at the level of the lower poles of the kidneys. The skin can then be retracted laterally toward either side and the ovary exposed through the thin muscle wall just below the dorsal muscle mass. Each incision should be of the minimum length which will conveniently allow the extrusion of the ovary. After hemostasis has been ensured by ligation of the upper horn of the uterus with silk, the ovary together with its surrounding fat, the oviduct, and a small portion of the uterus are excised. The wounds are closed with silk. If it is important that the uterus remain free from infection, asepsis must be maintained during the operation.

WINDOW TECHNIC FOR FETAL OBSERVATION

The description of this technic was taken directly from a paper by Gersoni.[8] A piece of sheet tin is cut having the dimensions 3.5 cm. x 4 cm. x 1 mm. A rectangular area is then cut out leaving a 4 mm. border. A sheet of clear, transparent celluloid is cut to the dimension of the outer frame (3.5 cm. x 4 cm.) and cemented to the frame with liquid solder. After drying, holes to fit the sewing needle are drilled through the tin and celluloid, ten holes on the long edge and seven on the short. The abdominal wall is cleared of hair by means of a chemical depilatory and painted with tincture of iodine. An area the size of the prepared window is completely excised from the abdominal wall and the defect is filled by sewing the window. The window should be sewed to the hide through the prepared holes in the frame by means of white silk thread, with the hair placed externally on the frame. If it is desired to observe a specific embryo it may be anchored by a loose ligature to the frame. Flexible collodion is then applied to the junction of the skin and window. The silk stitches in the frame are covered with liquid solder which may be replaced if damaged by the gnawing of the animal. If desired, a hole may be made in the celluloid window to admit a probe, and plugged with collodion when not in use. After a week the window becomes hazy due to the growth of scar tissue along its under side. In time the defect heals completely, the window comes off and the rat recovers.

UTERINE FISTULA

This technic was used by Florey and Walton[9] to study the ascent of spermatozoa in the female genital tract. The following description has been adapted directly from their paper. Aseptic precautions are used. The uterine horn is exposed by a median incision in the abdomen. It is secured by means of a retaining ligature passed through the mesentery. A short distance from the median incision and on the same side as the retained horn, a small incision is made. By means of the retaining ligature, a short segment of the uterus is pulled into the incision, care being taken to avoid distortion of the uterus or strangulation of any of the bowel. The large median incision is now closed. The segment of uterus is secured with catgut to the muscle coat and the retaining ligature removed. It is then opened by means of a longitudinal incision and the free edges stitched to skin with horse-hair. In a few days the lumen should be cleared of any clots or necrotic tissue. In about a week the skin has grown around the opening, leaving a small aperture large enough to insert a probe or small pipette. The aperture tends to close with time but can be kept open by passing a probe daily. The rat may then be mated when in estrus. Immediately after copulation undiluted semen containing masses of spermatozoa may appear at the open fistula.

LUMBAR SYMPATHECTOMY

The lumbar sympathetic chains lie directly in the midline posterior to the vena cava and abdominal aorta. The two chains lie in close proximity and often appear to unite at intervals, especially at the ganglia. Therefore, in most rats it is impossible to do an unilateral lumbar sympathectomy. If one chain is removed completely the other will be somewhat damaged. A long midline abdominal incision is made. The ganglia and chains may be exposed either on the right or on the left side of the great vessels. We prefer the left side because bleeding is minimized by this approach.

The intestines are wrapped in a wet, warm piece of gauze and are displaced to the right. The left kidney with its vessels and the cava and aorta are visible behind the peritoneum. The peritoneum is then torn over the aorta and cava to expose them more fully. Retraction of these vessels to the right is prevented by a large vein and smaller artery, lying a little below the renal vessels. These iliolumbar vessels must be ligated and divided. The aorta is then retracted to the right. This exposes the gulley formed by the deep spinal muscles. Small muscular venous branches leave the cava and pass to the left over these muscles. The sympathetic chain or chains can be identified as fine white strands passing over the redness of the blood-filled veins. The inexperienced operator may identify them more readily by using a magnifying loop. With a fine hook they may be picked up and dissected upward and downward. If it is desired to remove the chain behind the renal vessels the left kidney must be mobilized and displaced to the right. The dissection may be carried below the level of the bifurcation of the aorta into the pelvis, but this is difficult, especially in female rats.

The sympathetic chain is a very fine structure. It is best to dissect it free before attempting removal and then to remove it with forceps, if possible in one piece. Early in the dissection the genitofemoral nerve is encountered, a much larger structure which parallels the aorta. It may readily be identified by pinching, whereupon the scrotum or labium majus of the left side will retract.

EVISCERATION

For the removal of all of the intra-abdominal organs a two-stage operation is performed. In the first stage the right kidney is exposed through a ventral midline incision and the inferior vena cava is ligated between the points of entrance of the renal veins and the right lobe of the liver. Owing to the immediate circulatory changes induced by this procedure the adrenal glands may be injured. If deaths occur following this procedure, animals operated should subsequently be treated with maintenance doses of cortin for five days following the operation. We have found that the mortality rate of animals so treated is less than 10%. A period of 21 days should elapse between the first and second stage operations in order to permit the development of an adequate collateral circulation. In the second-stage operation a ventral midline incision is made extending from the level of the urinary bladder to the diaphragm, running to the left of the xiphoid process. The rectum with its vessels is clamped by means of a small hemostat, with ligation distal to the clamp, and then severed between the clamp and the point of ligation. The esophagus is severed in a similar manner at a point between the stomach and diaphragm. A single ligature is placed about the coeliac and the superior mesenteric arteries as near the aorta as possible. The gut is then freed by severing its ligamentous and venous attachments. The liver is freed from its ligamentous attachments and a linen ligature is placed about the vena cava at a point between the liver and the diaphragm. The cava is again ligated close to the kidney in order to close any capillary branchings which may have formed subsequent to the first ligation. The liver is then excised. The kidneys are excised after ligation of their vessels.

The adrenal glands should not be removed unless the experimenter desires to produce a state of adrenal cortical deficiency in the eviscerate animal. Before the adrenals are removed from the eviscerate preparation their vessels should be ligated.

Due to the absence of the liver the eviscerate animal develops a hypoglycemia very rapidly following operation. This can be prevented by the subcutaneous injection of 0.5 cc. of a 20% solution of glucose per 100 Gm. of rat every two hours. The animal should be kept at a temperature of 28°C. A range of survival times of 18-48 hours may be expected on the basis of our experience.

ORCHIDECTOMY

Using clean but not necessarily sterile technic, an incision is made in the tip of the scrotum large enough to permit the extrusion of the testicle. A single ligature is placed around the internal spermatic vessels, the deferential vessels and the ductus deferens. The testis, together with the epididymes are excised. If the incision in the scrotum has not been made unnecessarily large, it need not be closed by suture.

PARABIOSIS IN RATS

By W. A. Jeffers

We have used essentially the technic of Bunster and Myer.[10] Litter-mate male rats 20 days old, weighing about 20 Gm. are fasted for 12 hours prior to operation. Careful ether anesthesia is employed. As soon as the animals are anesthetized a depilatory paste is applied to adjacent sides of the two animals' bodies, allowed to act for 3 minutes, then removed. The skin surfaces are sterilized with 70% alcohol, and aseptic precautions are observed.

A skin incision is made along the midlateral aspect of each animal from the base of the ear to within 0.5 cm. of the base of the tail. With finest silk thread and a very small, straight, cutting-edge needle, the ventral flaps of each skin wound are sutured together with non-continuous sutures. The clavicles are then securely joined by placing a mattress suture of No. 12 braided silk, doubled, through them and their surrounding muscles, using a large, curved, cutting-edge needle. The adjacent abdominal muscles and peritoneum are then incised in the midlateral line, and the two pairs of raw edges are each joined together with a continuous fine, silk suture. A mattress suture of No. 12 braided silk is then placed through the gluteal muscles of the two animals to draw the caudal portions of the wound together. Finally, the dorsal edges of the skin wounds are united following the technic described for the ventral flaps. If very small "bites" are taken with the needle, it will not be necessary to remove the sutures later. At the anterior and posterior ends of the wounds an extra stay suture is placed to prevent separation of the skin edges.

The animals are kept warm until fully recovered from the anesthesia. They are allowed full diet postoperatively. After a week the skin sutures may be snipped in several places to allow better apposition of the skin to occur. Care must be taken to keep the cages dry, or infection of the wounds will occur.

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17

Histologic Methods Adapted for Rat Tissues

By

W. H. F. ADDISON

THE methods applied to the rat are in general the same as those used for the tissues of other mammals. One should remember in dissecting the rat that it is a small animal, and that its organs are correspondingly small. Therefore, the removal of tissues should be done rapidly in order to prevent drying and thus to avoid changes in the surface cells from evaporation. In the case of the smallest organs, such as the thyroid, the organs should be dissected under a magnifying glass. The connective tissue framework within the organ is less than in larger mammals and the penetration of the fixative and embedding fluids is consequently more rapid. Therefore, the time in these fluids is reduced.

When a permanent preparation is desired it is necessary to use a fixative to insure killing the cells rapidly. For most purposes, a general fixative is sufficient to give one an idea of the normal or pathologic condition of the cells. Some parts of the cells, as the nuclear membrane, are more resistant to deterioration than other parts and will persist after any fixation. Precision methods, however, are necessary to preserve certain structures, such as mitochondria and fat, which would otherwise be lost. For the purpose of demonstrating certain cytological constituents of the cell there are special fixatives which are to be followed by the appropriate stain. These special fixatives have to be applied with more care and often the staining requires considerable experience before satisfactory results are obtained.

GENERAL FIXATIVES

Pieces of tissue are removed in blocks 4-10 mm. thick and placed in the fixing fluid, or better the fixing fluid may be injected through the heart or aorta before the removal of the pieces to fixing fluid. Fixatives suitable for a general morphologic picture are formol, formol-alcohol, formol-Zenker, Bouin, and Susa. After fixation and removal of the fixative, tissues which are not to be embedded at once are stored in 80% alcohol.

Formol Fixative.

Formaldehyde, full strength	10 cc.
Distilled water or normal saline	90 cc.

This is the most convenient fixative, because of the ease with which it is made and its stability. Furthermore, it penetrates rapidly so that small organs or larger organs sliced to 4 mm. in thickness are preserved in two hours. It will penetrate larger masses, and, it will fix in 2 days an entire limb, if the skin has been removed, or a new born rat with the abdomen opened. Tissues may remain in the formol fixative for weeks or even months and still give reasonably good preparations.

From the fixative the tissues may be washed briefly in water or they may be placed directly into dehydrating alcohols—50%, 70%, 80% alcohol a day in each.

Formol-alcohol Fixative.

Formaldehyde, full strength	10 cc.
95% alcohol	90 cc.

This is also stable and cheap. Small blocks of tissue are fixed in this fluid 12-24 hours as convenient. They can be dehydrated without washing.

Formol-Zenker Fixative.

Potassium dichromate	2.5 Gm.
Sodium sulphate	1.0 Gm.
Mercuric chloride	5.0 Gm.
Distilled water	100 cc.

Dissolve salts by heating in water and allow to cool. Just before using add

Formaldehyde	5 cc.

Place small pieces of tissue in a generous amount of fixative for 10-24 hours. Wash in running water for 12-24 hours. Transfer to 50% alcohol for several hours. Dehydrate in 60%, 70% and 90% alcohols to which have been added a few drops of tincture of iodine which will remove the mercury crystals deposited in the tissues. Replenish the iodine as the color disappears from the alcohol. Six to twelve hours in each alcohol should suffice. Place the tissues in 95% alcohol to remove the iodine. If the tissues are to be stored before embedding return them to 80% alcohol. After the sections are cut and before they are stained place them in 95% alcohol, with iodine, for 3-5 minutes to ensure complete removal of the mercury deposits. Pass through 95% alcohol to remove the iodine before proceeding with the staining.

This fixative is especially good for cytoplasmic structures such as presecretion granules in glands and the granules in blood-formative cells.

Bouin Fixative.

Picric acid, saturated aqueous solution	75 cc.
Formaldehyde, c. p.	25 cc.
Glacial acetic acid	5 cc.

One gram of picric acid crystals will saturate about 75 cc. of water. It is convenient to keep ready a saturated solution of picric acid with an excess of crystals at the bottom.

Place the tissues in Bouin's fluid 4-24 hours. The tissues may remain in this fixative for a longer time without deterioration. Remove the fixative from the tissues by transferring them directly to 80% alcohol. If the alcohol is warm the picric acid compounds will come out more quickly. Change the alcohol until most of the color is removed. It is not necessary to complete the removal of the color at this time, but its removal does protect the dehydrating fluids which follow from contamination. The tissues are then ready for dehydration and embedding or they may be stored in 80% alcohol.

Bouin's fixative is especially good for the preservation of nuclear structures.

Susa Fixative.

Mercuric chloride	45 Gm.
Sodium chloride	5 Gm.
Distilled water	700 cc.

Dissolve salts in water by heating; allow to cool; add

Trichloracetic acid, 20% aqueous solution	100 cc.
Formaldehyde, full strength	200 cc.
Glacial acetic acid	40 cc.

Fix pieces of tissue 3-8 hours. Wash in several changes of 95% alcohol. Change to 95% alcohol to which are added a few drops of tincture of iodine. As the color disappears add more iodine to the alcohol. Place in clear 95% alcohol and change until the color of the iodine no longer appears.

If calcium is present in the tissues, some decalcification will take place during fixation. To further decalcify transfer tissues directly from the fixing fluid to 5% trichloracetic acid. Change the acid daily until trial with a fine sewing needle indicates that the tissue is decalcified. Transfer to 95% alcohol and proceed as above.

This fixative is very stable and rapid. After this fixation connective tissues fibers, epithelial cell boundaries, and muscle striations are well shown.

EMBEDDING

Embedding in Paraffin. If the tissues are in 80% alcohol, complete the dehydration by passing through 95% and absolute alcohols, a day in each. Then place in chloroform until they float below surface. The time varies from a few minutes to several hours, according to size and consistence. Remove to a previously prepared saturated solution of paraffin in chloroform. Leave overnight in a warm place or in oven not over 37 C. Change to melted paraffin of low melting point, 42-46 C, in oven for half an hour. Transfer to fresh paraffin of higher melting point, 54-58 C, two changes of an hour each. Block in fresh paraffin.

After paraffin sections are cut on the microtome, the sections are fastened to slides by albumen-glycerin mixture, and allowed to dry. Preparatory to staining, the paraffin is removed by xylol, 3-5 minutes, followed by absolute, 95%, 80%, 70%, and 50% alcohol, a minute in each alcohol, then into distilled water for a minute.

Rapid Method, Dioxan. The quickest method of embedding in paraffin is by the use of dioxan as the intermediate agent between the fixative and the paraffin. The method is thoroughly satisfactory except after silver impregnation. Dioxan is miscible in all proportions with water or alcohol and releases these substances to anhydrous calcium chloride. Because of this property when the tissue is taken from either a watery or an alcoholic solution and placed in dioxan containing calcium chloride the water or alcohol is removed and the tissue permeated with dioxan. Dioxan is also miscible with melted paraffin. Thus, when the tissue is transferred from dioxan to melted paraffin the dioxan is displaced and the tissue becomes infiltrated with paraffin.

In order to keep the fluid constantly free from alcohol and water a deep vessel is used in which there is a layer of anhydrous calcium chloride. Over this is placed a fine copper gauze on which the objects are placed. The vessel must be covered in order to prevent evaporation. Dioxan is readily inflammable and is irritating to the mucous membranes.

The tissue, after any fixation, is taken from the storage fluid, (alcohol, water, or formol) directly into the dioxan, where it may remain 3-24 hours as convenient, or even longer without injury to the tissue. From dioxan the tissue may be transferred into a mixture of dioxan and paraffin, 2:1, and kept in the oven at 55-60°C for 1-24 hours depending upon the size of the piece. This step is often omitted without any evident damage to the tissue or hindrance in cutting. The tissue is passed through three changes of pure melted paraffin in the oven and allowed to remain in each change 1-24 hours as desired. It is an advantage to keep the tissue on copper wire gauze when in pure paraffin. This keeps the tissue above the bottom where the dioxan gradually collects. The specimen is then blocked, cooled and is ready to section. The dioxan may be used repeatedly so long as it is not diluted too much. By this method, pieces of tissue 4 mm. thick may be readily carried from the fresh condition to the stained section in 48 hours.

Embedding in Nitrocellulose. Nitrocellulose embedding is necessary for larger blocks of tissue and it allows a great range in thickness of sections, 7-200 microns. The infiltration of the nitrocellulose is more gradual than is the infiltration of paraffin and because of this the nitrocellulose method preserves the natural shape and relationships of the cells. As in all infiltrations thorough dehydration is essential before embedding. Dehydration is accomplished by the use of graded alcohols. From 80% alcohol the tissue is placed in 95% alcohol for 24 hours. It is then placed in absolute alcohol with a layer of anhydrous copper sulphate at the bottom over which is a fine wire gauze on which to place the tissues. The length of time in this step, dependent upon the size of the block of tissue, is 24 hours or longer. The tissue is then placed for 24 hours in equal parts of absolute alcohol and ether with anhydrous copper sulphate as above. The tissue is now ready for infiltration with nitrocellulose which must be prepared some days in advance.

The stock solution of nitrocellulose is

Nitrocellulose (Hercules RS½)	100 Gm.
Ether	140 cc.
Absolute alcohol	100 cc.

Two to three days may be necessary before the nitrocellulose is completely dissolved. From this stock solution two dilutions are made with a mixture of equal parts of absolute alcohol and ether. The first dilution is one quarter strength and the tissues remain here for 24 hours. They are then removed to a half strength dilution for another 24 hours. Finally they are put into some of the stock solution where they remain for 2-3 days after which time the cover is slightly opened to allow the slow evaporation of the alcohol and ether and thus harden the nitrocellulose. When a crust forms on the top of the solution, it is customary to loosen the nitrocellulose from the edge of the container and to make cuts through the film so that evaporation may proceed evenly. When the nitrocellulose is firm throughout cover with 80% alcohol. When the nitrocellulose has been further hardened by the alcohol, cut out the blocks of tissue and store in 80% alcohol.

Embedding by Freezing. The most direct method of securing sections is by cutting frozen tissue. The tissues, after removal from the body, are placed in the fixative and, without further infiltration, are frozen and cut on the freezing microtome. This method is used in demonstrating fat and neuroglia. By placing the tissues in 20% alcohol for 24 hours before freezing with dry ice, the formation of ice crystals is inhibited (Wade H. Marshall, Stain Tech., 15, 133, 1940).

GENERAL STAINS

Certain stains as hematoxylin and eosin, Mallory's connective stain and iron hematoxylin are suitable after any of the fixatives mentioned before. Staining procedures are in general the same as for other mammals. The following are the methods as used in our laboratory.

Hematoxylin and Eosin. From water place sections in Ehrlich's acid hematoxylin for 12-15 minutes. The formula for stock solution of hematoxylin is as follows:

Hematoxylin	2 Gm.
Dissolve in absolute alcohol	100 cc.

Allow to stand in a loosely corked bottle in the sunlight for a week.

To 100 cc. of distilled water add ammonium alum in excess and add to hematoxylin solution. After several days add 100 cc. of glycerin and 10 cc. of glacial acetic acid. This solution must ripen in the sunlight from 4-12 weeks. Filter before using.

Rinse the sections in tap water and differentiate in acidulated alcohol, prepared by adding 0.5 cc. of HCl, c.p. to 100 cc. of 70% alcohol. Rinse in water and place sections in distilled water alkalized with a few drops of a saturated aqueous solution of lithium carbonate 5-10 minutes, or leave the sections in running water overnight. Counterstain in a 0.2% aqueous solution of eosin or erythrosin 15 seconds to 1 minute and rinse quickly in distilled water. Pass through 95% alcohol and leave in absolute alcohol 3-5 minutes. Clear in xylol and mount in balsam.

The nuclei stain blue; the cytoplasm, bone matrix, and collagenous tissue pink. Smooth muscle takes a slightly different color from the collagenous tissue.

Harris Hematoxylin. A more quickly prepared hematoxylin is that of Harris (a modification of Delafield's). One gram of hematoxylin is dissolved in 10 cc. of absolute alcohol; 20 grams of potassium alum are dissolved by warming in 200 cc. of distilled water. Next day mix the two solutions, and add to the mixture 0.5 gram of red or yellow mercuric oxide. Heat to boiling, then cool quickly by immersing the container in cold water. After 24 hours filter, and the stain is ready for use.

Mallory's Connective Tissue Stain. (Heidenhain's modification with azo-carmine.) This is a striking stain and gives sharp differentiation with thin sections. From 75% alcohol remove slides to a solution of 1 part aniline oil and 1000 parts 95% alcohol for 1 hour. Stock solution of 1:100 aniline-alcohol may be kept and diluted just before using. Stain in azo-carmine in oven at 45°C for 1 hour.

The stain is prepared by adding 1 gm. of azo-carmine to 100 cc. distilled water. Warm and allow to cool to room temperature. Filter.

Remove from oven and allow to stand at room temperature for 1 hour.

Differentiate in aniline-alcohol 1:1000 until the nuclei are red and the cytoplasm is faint pink. Mordant by placing slides in a 5% phosphotungstic acid solution for 1 hour. Wash quickly in distilled water.

Stain in aniline blue-orange G solution for 1 hour or more as determined after examination of the tissues under the microscope.

To preserve stain dissolve 0.2 Gm. of aniline blue in 400 cc. of distilled water and add 5 Gm. orange G. Heat until all stain is dissolved. Cool and filter.

Wash specimen quickly in distilled water and differentiate in 95% alcohol. Dehydrate in absolute alcohol, clear in xylol and mount in balsam.

The nuclei and erythrocytes are stained red; the collagenous tissue and neuroglia blue, and the muscle red or orange.

Heidenhain's Iron Hematoxylin. This is an excellent cytological stain and is used extensively for chromosomes. From distilled water the sections are taken to a 4% aqueous solution of iron ammonium alum and left for ½-24 hours. This acts as a mordant. Wash in water. Stain in 1% aqueous hematoxylin ½-24 hours.

A saturated solution of hematoxylin should be made up in absolute alcohol and 1 cc. of this is diluted with 99 cc. of distilled water before using. The stain may be reused. After staining wash in water.

Differentiate in 1% or 2% iron ammonium alum. Watch differentiation carefully under the microscope and dilute the differentiator if the action is too rapid. Washing thoroughly, 15-30 minutes, in running tap water stops the differentiation. Counterstaining is not necessary, but, when desired, eosin may be used.

After proper differentiation the nuclear and certain cytoplasmic structures remain black against a pale background.

FIXING AND STAINING OF SPECIAL STRUCTURES

For the demonstration of certain structures which are not preserved by the preceding fixatives or shown after the foregoing stains the following special procedures are applicable.

TABLE XXIII

To show	*Fixative*	*Stain*
I. a. presecretion in non-mucous gland cells	formol-Zenker	neutral gentian
b. presecretion in mucous gland cells	alcohol	mucicarmine
II. Granules in blood-formative organs	formol-Zenker	Azur II-Eosin
III. Fat	acetic dichromate osmium tetroxide	Sudan III
IV. Glycogen	alcohol	iodine; carmine
V. Component parts of neurons		
a. myelin sheaths		
1. of peripheral nerves	osmium tetroxide	
2. in central nervous system	10% formol	iron hematoxylin
3. degenerating myelin	potassium dichromate	Marchi fluid
b. axons	ammoniated alcohol	silver nitrate
c Nissl substance	alcohol; formol	aniline stains
VI. Neuroglia, astrocytes	formol-ammonium-bromide	gold chloride
VII Microglia	formol-ammonium-bromide	silver carbonate
VIII. Mitochondria	formol-dichromate	iron hematoxylin
IX. Golgi apparatus	osmium tetroxide osmium sublimate	
X. Chromosomes and Centrosomes	Bouin 15 (Allen)	iron hematoxylin
XI. Elastic Fibers	any general fixative	basic fuchsin

To Show Presecretion in Non-Mucous Gland Cells. Fix pieces of glands in formol-Zenker which preserves many granules well. Stain sections 24 hours in the gentian violet-orange G stain of Bensley.

This is a neutral stain prepared by adding a filtered saturated aqueous solution of orange G to a filtered saturated aqueous solution of gentian violet until a precipitate forms. Collect precipitate by filtration and dry in oven at 37°C. Dissolve the precipitate in absolute ethyl alcohol to a point of saturation. Before using, dilute 5 cc. of the stock solution with 45 cc. of 20% alcohol made from absolute alcohol.

Blot sections on filter paper and wash quickly in anhydrous acetone. Transfer to benzol. Differentiate in a solution of absolute alcohol, 10 cc. and oil of cloves, 30 cc. Watch differentiation under the microscope. Transfer to benzol. Mount in balsam.

The presecretion and the red blood cells are stained purple, while the general cytoplasm and nuclei are stained yellow. This stain is particularly good for the pancreas and the stomach. For the hypophysis Mallory's connective tissue stain has been satisfactory.

To Show Presecretion in Mucous Gland Cells. The following method is selective. Fix pieces of tissue in 70% alcohol for 24 hours, using several changes during that time. Dehydrate and embed in paraffin. After staining with hematoxylin bring the sections into 70% alcohol and stain in mucicarmine for 5-10 minutes.

Prepare the mucicarmine as follows:

Mucicarmine	1 Gm.
Aluminum chloride	0.5 Gm.
Distilled water	2 cc.

Heat over a small flame for 2 minutes, add 50% alcohol to make 100 cc. This stain may be used undiluted or may be diluted 10 times with water. Pass directly from the stain into 95% alcohol. When all excess stain has been removed, place sections in absolute alcohol for 2 minutes. Clear in xylol and mount in balsam.

The nuclei will be stained blue, and the mucous secretion red.

To Show Granules in Blood-Cells of Blood-Formative Organs:—Bone Marrow, lymphatic organs. Fix in formol-Zenker and embed in nitrocellulose or paraffin. Affix the sections to the slides. If embedding was in nitrocellulose remove all nitrocellulose from the slide. After removing mercury deposits from sections wash well in distilled water. Great care must be taken to keep alcohol out of the dye as it precipitates the dye at once.

There are two stock solutions of the stain:

A. Azur II (Grübler) 1:1000 in distilled water
B. Eosin, water soluble 1:1000 in distilled water

Place slides in the following stain for 12-24 hours:

Solution A	10 cc.
Solution B	10 cc.
Distilled water	80 cc.

Differentiate in 95% alcohol, pass through absolute alcohol and xylol and mount.

To Show Fat. Fix thin pieces, 1-2 mm., of fresh tissue 24-48 hours at 45°-50°C in the following solution:

10% aqueous potassium dichromate	100 cc.
Glacial acetic acid	5 cc.

Wash in running water 12-24 hours. Dehydrate and embed in paraffin. Cut sections 3-5μ; fasten to slide; remove paraffin with xylol and transfer to absolute alcohol. Transfer directly to freshly prepared solution of Sudan III for 10 minutes. Prepare a saturated solution of Sudan III in 80% alcohol by heating over a water bath. Cool at room temperature, and filter.

Rinse off excess stain in 70% alcohol, transfer immediately to water to stop action of alcohol. Mount in glycerin gum arabic (5 Gm. gum arabic in 5 cc. distilled water. Let stand 12 hours and then add 5 cc. glycerin and 10 cc. of 5% carbolic acid.)

Tissues may be counterstained a few minutes in hematoxylin and differentiated in acid alcohol, either before or after staining with Sudan III.

This method has been used to show lipoid droplets in the cells of the cortex of the adrenal. The fat will be colored red.

Pieces of omentum containing fat cells are stained by simple immersion in the above Sudan III solution for 5-15 minutes; likewise frozen sections.

Unsaturated fat may be demonstrated by treatment of the tissues with osmium tetroxide. Very small pieces of tissue are immersed in a 1% solution of osmium tetroxide for 24 hours. Wash thoroughly. Tease apart and examine directly under the microscope, or cut after embedding in paraffin and mount without further staining. The osmium tetroxide penetrates very slowly and the sections will be of different intensities as one proceeds through the block of tissue. The fat will be black.

To show glycogen. Fix small pieces of tissue in 95% alcohol. Transfer to two changes of absolute alcohol for 24 hours or longer. Embed in paraffin and cut sections 10-15μ. Prepare sections for staining by affixing them to the slide with stain or with alcohol instead of water. Avoid melting the paraffin.

The stain is prepared as follows:

95% alcohol	150 cc.
Distilled water	150 cc.
Iodine crystals	1.5 Gm.
Potassium iodide	3.0 Gm.
Sodium chloride	1.5 Gm.

After 5 minutes pour off stain, wipe around the section and allow it to dry thoroughly in air. Remove paraffin in xylol and mount in slightly warmed vaseline. The glycogen will be brown.

An alternative method is using Best's carmine stain, which is most successful on material embedded in nitrocellulose after alcoholic fixation. The sections may be stained in hematoxylin before proceeding with the carmine.

Prepare the stock carmine solution as follows:

Carmine	2 Gm.
Potassium carbonate	1 Gm.
Potassium chloride	5 Gm.
Distilled water	60 cc.

Boil gently for a few minutes; cool at room temperature. Add 20 cc. of strong ammonium hydroxide. This solution is not stable and may deteriorate rapidly in warm weather. Keep in a well stoppered bottle.

The formula for the stain is:

Stock carmine solution	20 cc.
Strong ammonium hydroxide	30 cc.
Methyl alcohol	30 cc.

From distilled water put sections into the stain for 5 minutes. Differentiate in the following solution 3-5 minutes, or until the carmine no longer leaves the tissues.

Absolute alcohol	40 cc.
Methyl alcohol	20 cc.
Distilled water	50 cc.

Wash in 80% alcohol, pass through 95% and absolute alcohols. Clear in xylol and mount in balsam. The glycogen will be red.

To Show Component Parts of Neurons. Myelin sheaths of peripheral nerves are prepared as follows.

Immerse nerve in 0.5% aqueous solution of osmium tetroxide. Twenty-four hours should be allowed for the osmium tetroxide to penetrate thoroughly. The penetration is slow, but if the immersion is continued for too long a period the nerve will become brittle. Wash nerve in running water for 12-24 hours. The nerve may be studied after teasing the fibers apart or the nerve may be dehydrated, embedded, and sectioned. The myelin will be blackened.

Myelin sheaths in the central nervous system can be prepared as follows. Fix tissue in 10% formol, cut sections 15-30 microns, and wash in distilled water. Mordant the sections in 5% aqueous solution of potassium dichromate from five to ten minutes. Wash in two changes of tap water. Stain from 15-30 minutes in the following solution kept in the oven at 48-50°C:

4% aqueous solution of ferric ammonium sulphate	50 cc.
1% solution of hematoxylin	50 cc.

The hematoxylin is made by adding 45 cc. of distilled water to 5 cc. of 10% solution of hematoxylin dissolved in absolute alcohol, freshly dissolved and not filtered. Wash in two changes of tap water. Differentiate in 4% aqueous solution of ferric ammonium sulphate until the gray matter is distinguishable. Wash in three changes of tap water. Complete the differentiation in the following solution:

Potassium ferricyanide	2.5 Gm.
Sodium biborate	2.0 Gm.
Distilled water	200.0 cc.

Wash in two changes of tap water. Place sections for 30 seconds in 100 cc. of tap water to which have been added 6 drops of concentrated ammonium hydroxide. Wash thoroughly in tap water. Dehydrate starting at 95% alcohol. Clear in xylol and mount.

For staining myelin sheaths in the central nervous system the procedure of Weil as given above is used almost exclusively in this laboratory. The stain may follow either paraffin or nitrocellulose embedding. Good results are obtained by staining nitrocellulose sections before mounting on the slides. The myelin takes a deep blue color and the background is gray.

Degenerating myelin within the central nervous system may be stained by the following procedure. Fix entire brain in 3% aqueous solution of potassium dichromate for 4 days. Cut out parts desired and place in fresh potassium dichromate for 5-10 days. Slices should be thin, not over 4 mm., to allow penetration of fluid. Place pieces directly into the Marchi fluid where they remain for one to two weeks. Keep in the dark in a glass-stoppered bottle. Change to fresh fluid after one week. The Marchi fluid consists of 50 cc. of 3% aqueous solution potassium dichromate and 25 cc. of 1% solution of osmium tetroxide.

Wash in running water from 24-48 hours until the odor of the osmium tetroxide has disappeared. Dehydrate starting with 50% alcohol. Embed in nitrocellulose. Cut sections from 20-25 microns. No further staining is necessary. The degenerating myelin will show as black dots and flakes on a yellowish or brownish background. Special care must be taken in handling the fresh tissue in order not to displace the myelin.

Material is usually taken between the tenth and twenty-fifth day after the operation. Fourteen days after enucleation of the eye of the rat degenerating fibers were seen in the lateral geniculate body.

There are many methods and variations for the study of the axons. The modification of Ranson is much used in studying the unmyelinated and the myelinated axons. The procedure is as follows:

Fix pieces of nervous tissue in absolute alcohol with 1% ammonium hydroxide for 48 hours. Rinse in distilled water and refix in pyridine for 24 hours. Wash in many changes of distilled water for 24 hours. Place in 2% aqueous solution of silver nitrate in dark at 35°C for 3 days and then rinse in distilled water. Reduce for 24 hours in

Pyrogallol	4 Gm.
5% formol in distilled water	100 cc.

Wash in distilled water. Dehydrate and embed in paraffin.

The axons of both myelinated and unmyelinated nerve fibers are black and the surrounding tissue golden brown.

Nissl substance may be fixed by the following procedures. Fresh tissues may be fixed in either 95% alcohol or 10% formol for 1-2 days. Paraffin embedding is preferred. Stain for 1-18 hours in a 0.1-1% aqueous solution of toluidine blue, methylene blue, or cresyl violet. Longer staining gives more permanent results. Rinse in water. Dehydrate and differentiate in 80%, 95%, and absolute alcohol. Clear in xylol and mount in balsam. The Nissl substance will be stained blue. Counterstain with eosin if desired.

To show neuroglia and astrocytes, remove blocks of brain not over 5 mm. in thickness immediately after death of rat and place in the following formol-ammonium-bromide solution for 4-24 days at room temperature.

Formaldehyde, c.p. full strength	14 cc.
Ammonium bromide	2 Gm.
Distilled water	86 cc.

Cut sections on the freezing microtome at about 25 microns and place directly into distilled water to which a few drops of formaldehyde have been added. Pass the sections rapidly through two changes of distilled water and place in stain, freshly prepared as follows:

Mercuric chloride crystals	0.5 Gm.
Gold chloride (brown, Merck) 1%	10 cc.
Distilled water	50 cc.

Pulverize $HgCl_2$ crystals and dissolve rapidly without overheating. Add gold chloride solution while water is still hot. Filter the mixture. The gold chloride solution will keep for months in a brown bottle. All glassware used in the making of these solutions and in the procedure to follow must be scrupulously clean.

Sections must not overlap in the staining solution. Keep sections in the stain in the dark 4-6 hours. Sections will turn an intense purple when staining is complete. The astrocytes and their processes will be a dark purple.

Transfer to the following fixing bath for 6-10 minutes

Sodium hyposulfite	5 Gm.
Water	70 cc.
95% alcohol	30 cc.
Concentrated solution of sodium bisulfite	5 cc.

Wash sections in 50% alcohol, and mount on slide. Dehydrate with absolute alcohol. Clear in oil of origanum, then in xylol, and mount in balsam.

To Show Microglia. Blocks of tissue may be fixed as for astrocytes for 2-3 days at room temperature. Place blocks in fresh formol-ammonium-bromide solution in a small covered glass dish and heat to above 50°C for ten minutes. Cut sections at 20-25μ on the freezing microtome. Wash sections in three changes of distilled water to the second of which is added 4-5 drops of ammonium hydroxide. Place four or five sections in Hortega's silver carbonate solution prepared as follows:

10% silver nitrate	5 cc.
5% sodium carbonate	20 cc.

Add fresh ammonium hydroxide drop by drop until the precipitate is just dissolved. Add distilled water to make 75 cc. Filter and keep in a dark bottle. Remove one section after 20 seconds, another after 45 seconds and a third after 2 minutes. Plunge them directly into 1% formol for 1 minute. Here the sections should change to a gray color. After the optimum duration of silver impregnation has been determined under the microscope a number of sections may be carried through at one time. Wash sections in distilled water.

Tone sections in the following solution until they become an even gray color:

Gold chloride (yellow)	1 Gm.
Distilled water	500 cc.

Fix the sections by placing them in a 5% solution of sodium hyposulfite for 1 minute. Wash sections in water, dehydrate on the slide. Clear in carbolxylol-creosote mixture and cover. (The clearing mixture is creosote, 10 cc., phenol, 10 cc. and xylol, 80 cc.) The microglia will be black.

To show mitochondria. Fix for 3 days in the following fixative:

3% potassium dichromate	80 cc.
Neutral formaldehyde	20 cc.

Remove to 3% potassium dichromate. Change every other day for 6 days. Wash tissues for 24 hours preliminary to embedding in paraffin. Heidenhain's iron hematoxylin following this fixative gives exceptionally good staining of mitochondria.

To Show Golgi Apparatus. Fix small pieces of tissue in 2% osmium tetroxide for 9-14 days in the dark. Wash in running water for several hours. Dehydrate, embed in paraffin and cut thin sections.

A second method is to fix in osmio-sublimate fluid ¼-3 hours. Prepare a fresh mixture of:

1% osmium tetroxide	50 cc.
Saturated solution of mercuric chloride in 0.9% solution of sodium chloride	50 cc.

Wash in two changes of distilled water for ½ hour. Cover with 2% osmium tetroxide for 10-14 days. Do not let osmium tetroxide evaporate and replace it if it loses its characteristic odor. Transfer to water at 38°C for 24-48 hours. Wash in running water. Dehydrate, and embed in paraffin. Cut thin sections. The Golgi apparatus will be blackened.

To show Chromosomes and Centrosomes. Fix tissues in B15 (Allen), a modification of Bouin's formula.

Picric acid, saturated aqueous solution	75 cc.
Formaldehye	25 cc.
Glacial acetic acid	5 cc.

To the above solution, the original Bouin's fluid, add 1.5 grams of chromic acid. When this has dissolved, add 2 Gm. of urea. After fixation remove the picric acid with alcohol as described under the preparation of Bouin fixative. Heidenhain's iron hematoxylin is recommended to show chromosomes and centrosomes after this fixation.

To Show Elastic Fibers. Stain sections for 10 minutes, or longer, in basic fuchsin in a covered dish. Basic fuchsin stain is prepared as follows:

Distilled water	100 cc.
Basic fuchsin	1 Gm.
Resorcin	2 Gm.

Heat in a glass or porcelain dish until it boils. Add 12.5 cc. of 30% aqueous solution of ferric chloride. Continue to boil, stir frequently for 5 minutes. Allow to cool; filter; wash precipitate with distilled water several times. Dry precipitate on filter paper and then place in 100 cc. of 95% alcohol. Heat carefully until the precipitate is dissolved in the boiling alcohol. Allow to cool, add 95% alcohol to make 100 cc. Add 2 cc. of hydrochloric acid. This staining solution can be kept for years and re-used.

Differentiate the sections in 95% alcohol, several changes. Sections may be destained, if necessary, in ½% hydrochloric acid in 80% alcohol. The elastic fibers are stained deep purple or black, and other tissues are nearly colorless. The staining of the elastic fibers may be followed by staining with hematoxylin and eosin to complete the picture.

Other methods of histological technic are given in larger books such as McClung's *Microscopical Technique* and Bensley's *Handbook of Histological and Cytological Technique.*

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18

The Osseous System

By

R. M. STRONG

THE methods described in this chapter have been selected to meet the needs of investigators who may wish to study the skeleton of both normal animals and rats which have been used in experimental studies. The technic of experiments which may affect the skeleton has not been included. The reader is referred to the chapter on radiology for x-ray technic and to the large literature involving rachitis, nutrition, growth, etc., for methods employed in work on these subjects. The preparation for study of osseous structures of rats which have been involved in such work is considered, however, in this section.

METHODS FOR PREPARING GROSS SPECIMENS

Dry Skeletal Material

Various methods have been described for the elimination of overlying soft parts. According to my experience, it is desirable to avoid any procedure which involves much putrefaction because of unpleasant dissecting conditions and lingering odors. Also more care is required if the ligaments are not to be removed.

If it is not convenient to proceed directly with cleaning of the skeleton, the animal should be placed in either alcohol or formalin (a 5% solution of formalin is satisfactory). The writer prefers alcohol. Large specimens should have an opening made in the abdominal wall to facilitate penetration of the fluid. Eventually, the integument and viscera should be removed. More or less muscle should also be dissected away in the case of large rats, i.e. over six weeks or so old.

Preparation of Bones, with Ligaments and Periosteum Removed. Where single detached bones are desired, some macerating method should be used:

Gold-dust Washing Powder Method.

The following technic[1] has been used successfully by various investigators. This involves the use of "Gold Dust Washing Powder" which has the following approximate composition. Sodium carbonate, 45 per cent; soap powder, 30 per cent; water, 25 per cent.

The partly dissected rat is placed in 50 to 200 cc. of a hot Gold Dust Solution in tap water. For mature rats a 2% solution at 96°C is used for an hour, more or less, the material being watched and tested. The object to be attained is a complete softening of the attachments of the muscles, so that they may be readily removed with the bone scraper, or brush used for cleaning the bones. The younger the rat the weaker should be the solution, the lower the temperature used, the shorter the time, and the more careful the watching.

Antiformin Method. Another method which would seem to have great advantages has been described by Green.[2] It is based on a technic first published by Johan (1924), for fresh human material. According to Green, it is available for small mammals and may be used for material preserved either in alcohol or formalin as well as for fresh tissues. In fact it gives even better results with fixed material. The expense is stated to be "almost negligible," and "clean specimens may be obtained within an hour from any sort of material."

Specimens from which the main masses of soft tissue have been removed are placed in an antiformin solution which has the following composition in concentrated form.

Sodium carbonate	150 grains
Bleaching powder	100 grains
Tap water .	1 litre

The sodium carbonate is put into ¼ litre of water, and the bleaching powder into the remaining ¾ litre. The two solutions are then mixed and shaken for three to four hours followed by filtering. An equal quantity of a 15% sodium hydroxide solution is added to the filtrate.

For fresh tissues, one part of antiformin and eight to ten parts of water are used. With fixed material, the proportion is one part of antiformin to about five parts of water. The diluted antiformin solution is heated to "just short of boiling" and is kept at this temperature.

After a few minutes, the material should be examined to see whether maceration has proceeded far enough to loosen soft tissues. If the bones are not nearly clean, the specimen should remain under observation until most of the soft tissue has been removed, when it should be taken out of the solution. Fragments of soft tissue still adhering may be removed easily with a brush.

If left too long in the solution, the bones, particularly the very small ones, will start to disintegrate.

The cleaned bones are then washed in running water and dried. The antiformin solution may be used over again several times, but should be filtered each time, and it may be desirable to add a little fresh antiformin. Bones thus prepared are said to be clean and almost free of fat. If there is any tendency to surface flaking of bones, a much more dilute solution of antiformin should be used with a longer period of maceration.

Potassium (or Sodium) Hydroxide Method. Maceration of material which may have been preserved for a long time in alcohol or formalin can be done easily in two to five % solutions of potassium (or sodium) hydroxide in water. Weaker solutions are used for very young rats. The period required varies from a few days to a few weeks, depending upon the age of the rat and the strength of the solution employed.

After a day or two, the material should be examined daily to note the progress of the maceration. When considerable clearing of soft tissue has occurred, the specimen should be tested with a pair of forceps. If flesh is easily removed, the specimen should be washed in tap water. The bones can then be cleaned and dried.

This method is simple, and the clearing that occurs is advantageous in locating small bones. It is recommended especially for young rats.

Ward Method. A method recommended for preparing cat skeletons. (See Ward Nat. Sci. Bull., 1936, **10:** No. 4.) It is presumably adapted to the preparation of skeletons of adult rats. As much flesh as possible is dissected away from the skeleton, using care not to cut the cartilage or bone. The specimen is then placed in a two per cent solution of household ammonia in tap water for two or more days.

A solution which consists of two parts commercial chlorinated lime, one part sal soda and eight parts tap water is heated to the boiling point, and this is brushed onto the skeleton until all flesh has been removed. Hands and clothing should be protected from this solution as from the other caustic solutions mentioned. The skeleton is placed in cold water and then drained.

Scavenger Method. A method used by Phillips[3] for cleaning fish skeletons could be considered for removing soft tissue from rat skeletons when the necessary scavengers are available. He states that the skeletal parts "were cleaned quickly and efficiently by enlisting the aid of the marine isopod *Cirolani harfordi,*" which was abundant at the Hopkins Marine Biological Laboratory beach when the work was done.

Bleaching Methods. If white specimens are desired, the bones should be placed in a hydrogen peroxide solution consisting of one part commercial hydrogen peroxide to twelve or more parts of water. If sufficient bleaching has not occurred in a day or two, the bones can be left in the solution longer.

Fat Removal. Fat may be removed with benzine or benzol.

Rat bones are soaked about three days in benzine or benzol following thorough dehydration. Ether is used for ground sections.

Drying. The following drying method has been described by Jackson[4] The rat bones are dried in a dust-proof case at ordinary room temperature for thirty days followed by several days in an oven at about 90°C.

Preparation of Entire Skeleton Intact or Mounted. When it is desirable to keep the skeleton intact, either the ligaments must be retained to some extent, or a laborious system of assembling and bone-wiring must be employed. A simple procedure, when a fine museum specimen is not needed, involves stopping maceration in one of the preceding technics just before the joints begin to loosen. According to my experience, the potassium (or sodium) hydroxide method works well. This involves careful daily examination to determine when soft tissues are sufficiently macerated to be removed easily without losing all ligamentous connections of bones at joints. Ordinarily, only daily examinations are necessary.

When the specimen is sufficiently, but not too much macerated, soft tissues may be removed with forceps and a tooth brush. Care is of course necessary to avoid separations of bones at joints. Washing in tap water and drying follow.

If a fine museum specimen is desired, bleaching, wiring and mounting will be necessary. This work is easier, if maceration has not completely destroyed the ligaments. Such a specimen when still wet from the hydrogen peroxide bleaching solution should have enough ligamentous tissue left to preserve joint relations. If the skeleton has become dry, it should be relaxed in water.

For further details, the reader is referred to the Ward Natural Science Bulletin or to works on museum methods.

Cleared Specimens

Clearing in Potassium (or Sodium) Hydroxide. The writer[5] has used this method successfully for fetuses and young rats up to two months. The specimens are fixed in undiluted ethyl alcohol in which they should remain for several or more days or weeks. The period can be much shortened by using absolute alcohol after thorough penetration has been secured. Further treatment is as follows. They are then carefully skinned and eviscerated. Even the tail and feet are skinned. The brains are removed from rats more than a week old. The specimen is then placed in a solution of potassium hydroxide. For young fetuses, when ossification is first appearing, a 1% solution is employed. In the case of older material, the strength is increased.

As has been my experience with chick embryos, there is considerable individual variability in the time required for clearing, even when the treatment has been uniform. Occasionally a specimen clears with difficulty and may take weeks or months with a treatment that is successful for other individuals of the same age in the course of a week or two. I have not been able to account for this variability. In all cases, it has been necessary to watch the specimen and make changes as clearing appeared to progress.

In general, I have found it most satisfactory to transfer very young specimens, after a few hours in potassium hydroxide, to a solution used by Mall.[6] This solution consists of 1% potassium hydroxide, 20% glycerin, and 79% water. Here they stand for a week or even months, according to the progress of the treatment and convenience. They are then brought back to a 3% solution of KOH, or 5% with rats more than one week old. Specimens must be examined daily to determine when they are ready for the transfer to glycerin or are in danger of maceration. Some specimens have had a second treatment in Mall's solution. The specimen sometimes becomes remarkably clear in Mall's solution and can be transferred directly to glycerin.

Except in very young stages, both with bird and rat material, troublesome accumulations of opaque white material appear in a number of places. I have not found a feasible solvent for these masses, and it has been necessary to dissect them away—a tedious and time-consuming process. I am indebted to Dr. R. M. Hill for an analysis of this material which he reported to be a partly saponified fat. By removing fat-masses in the superficial fasciae, the amount of the formation is decreased. There still appear, however, deposits in regions where it is especially difficult to dissect them away. The deposits are most conspicuous when the specimens are in potash solutions and are less so in glycerin. Though less opaque in glycerin, they are serious obstructions to clear views and must be removed.

Dawson[7] found it possible to get rid of this fat nuisance by the following treatment. After thorough fixation in 95% alcohol, the specimen is "transferred directly to acetone, being left there for several days, depending on the bulk of the object being treated. Following this treatment, the specimen is transferred directly back to 95% alcohol for twenty-four hours or longer."

I have not found it an advantage to use Miller's graduated alcohol and glycerin stages with specimens as small as the rat, nor has a cartilage stain been a help in my work. Material thus treated was, in fact, injured for my purpose. There was considerable decalcification, which rendered specimens, in early stages of ossification, useless.

Many preparations had a brown stain, which was successfully removed by immersion in hydrogen peroxide for one or more days. Numerous bubbles resulted, which were removed by aspiration. It was usually necessary to use a rather strong electric air pump for periods of several hours. Bubbles of gas would emerge from all parts of the specimen, often in rapid succession, but no damage from their breaking out was observed.

Various microscopical combinations were tried, including a prism binocular dissecting microscope, for making observations. By far the most satisfactory apparatus was a simple dissecting microscope having a glass stage and a single lens mounted in a movable arm. Reflected light was often employed in checking observations of beginning ossification by transmitted light. Oblique illumination effects, obtained by simply holding one hand in varying positions on the light side of the microscope, were sometimes useful. Manipulation of the substage mirror was helpful, occasionally. The best results were obtained when strong sunlight was available—even direct sunlight being advantageous in some cases—but a low desk light made a good substitute on dark days. Calcified cartilage is usually easy to distinguish. It has a granular appearance under the dissecting lens, and it is commonly more or less invisible when transmitted light is employed. The trabeculae and spicules of developing bone have a characteristic appearance. At the beginning of my work, I was often in doubt as to whether real bone tissue was present or not. Eventually, it became possible to determine beginning ossification at a glance.

Stender dishes have been found the most convenient containers both for storing and for examination. Some of the photographs were made by using a 32-mm. focus moving-picture lens, but a 5-inch lens with a long bellows gave the best results. Cleared specimens were photographed by transmitted light. As the resulting negative would make a print with the bone centers dark, a positive plate was made. This method causes skeletal elements to appear white. Early stages of membrane bones, where very thin, did not photograph well, though clear to the eye. The wings of the palatines are especially incomplete in the photographs.

Staining with Madder or Extracts from It. It has been known for centuries that madder, when ingested, will stain developing-bone red. For histories and bibliographies of the subject, see Cameron,[8] Gottlieb,[9] and Ercoli and Lewis.[10] One of the extracts is alizarin.

(1) Alizarin Red S. Staining Methods. Calcifying cartilage can be stained, and I recommend a method by Dawson,[7] who has used it with great success for leg bones of rats from one to thirty days of age. The legs are fixed in 95% alcohol for at least from 48 to 72 hours, depending on the age of the animal. Longer fixation is advantageous, i.e., weeks or months.

The skin is removed carefully, and the specimen is then placed in a 1% solution of potassium hydroxide for from 24 to 72 hours or until the bones are clearly visible through the muscle tissue. The specimen is then placed in a solution consisting of one part alizarin red S(alizarin mono-sodium sulphonate) to 10,000 parts of 1% potassium hydroxide, where it remains until the bones are stained the proper color. "If the dye is absorbed from the solution before the maximum intensity is obtained, the specimen may be transferred to a fresh solution of stain."

When the staining is completed, the material is placed in Mall's clearing solution.[6] When sufficiently cleared, the specimen is passed through increasing concentrations of glycerin and stored in pure glycerin.

If clearing in the initial potash solution has been adequate, only the bones will be stained. However, if the clearing has not been sufficient, the muscles and other soft tissues will also be stained.

A more rapid method has been described by Richmond and Bennett.[11] This involves a much more concentrated stain. After the initial clearing, as described above, the specimen is placed for from 30 to 60 minutes "in a freshly-prepared 0.1% aqueous solution of alizarin red S (alizarin mono-sodium sulphonate) to which 6-10 drops of 1% KOH are added." It is then washed for 30 minutes in running tap water and is decolorized in Mall's solution,[6] as in Dawson's method. "The KOH is reduced to 0.5% for small specimens." Further treatment is similar to that described by Dawson except that there is a dehydration. A series of grades of alcohol and glycerin are used.

The Nobacks[12] cleared and stained human embryos and fetuses simultaneously in a solution of alizarin red S in aqueous KOH (preferably in distilled water) to avoid the danger of maceration. This method should be applicable to rat material. However, maceration does not occur if clearing in KOH is not too prolonged.

According to Lipman,[13] decoloration can be done in acid alcohol (1% H_2SO_4 in 95% alcohol).

(2) Vital Staining Methods. (a) Method of Ercoli and Lewis.[10] Ercoli and Lewis used intravenous injections of alizarin red S with mice. A single injection of from 20 to 30 mg./kg. of this dye caused bone coloration in growing mice. Adult mice required from two to three times this amount. They concluded that fixation occurs by a process of adsorption between existing calcium phosphate and dye anions rather than by the supposed formation of calcium alizarate or a reaction with free calcium ions.

(b) Macklin's Method.[14] A small quantity (from one to five grains for each adult animal) is mixed thoroughly with the food and is fed daily. The stain appears rapidly in the calcified cartilage. The material is fixed in 10%

neutral formalin and then washed and dehydrated. Gross specimens are cleared by either the potassium hydroxide or Spalteholz methods.

Richter[15] has published a detailed description of the chemical and dye composition of madder. He found in vital staining of bones with madder, that purpurincarboxylic acid present in madder is the important red dye and not the alizarin present.

Rat bones were stained in the following solution for four hours at 37°C. "24 mg. purpurincarboxylic acid were ground with a slight excess of NaOH in 10 ml. water and the solution was neutralized with 5% acetic acid. To this was added (a) a solution of 0.5 gm. gelatin in 50 ml. water, (b) a solution containing 0.18 gm. NaCl, 0.046 gm. $CaCl_2$ and 0.05 gm. KCl, and 20 ml. M/1 buffer solution. The mixture was filtered and diluted to 200 ml." The staining experiments gave similar results at pH 4.6 (acetate buffer) and 6.2 (phosphate buffer). Bone was deeply stained by the method, especially when young. Calcium phosphates were strongly stained, but crystalline apatite, calcite and calcium carbonate were "hardly stained at all."

Spalteholz Clearing Method.[16] I do not recommend this method except where it is desirable to show other structures in addition to bone. Rats are fixed in 80%, 95% and absolute alcohol for at least 72 hours each. They are then placed in benzine (or benzol) for 72 hours followed by a mixture of benzine and methyl salicylate (synthetic oil of wintergreen) equal parts for 72 hours. From this mixture they are passed to pure methyl salicylate and allowed to stand for about a week until the clearing is satisfactory. Then they should be transferred to a fresh solution of methyl salicylate for permanent storage.

Formalin has been used by some workers instead of alcohol for fixation but we do not find it so satisfactory.

An undesirable brown stain may occur if specimens are not prepared in separate containers. Air bubbles can be removed as described for potassium hydroxide cleared material.

Developing bone can be stained advantageously in specimens to be cleared by the Spalteholz method, according to Shipley and Macklin.[17]

"If an azo dye (e.g., trypan blue) be administered to a very young animal the bones are stained quickly and very intensely with the vital color. The staining is so rapid and selective that the growing osseous system is colored blue black throughout its entire extent, whereas the organs (the liver, spleen, etc.), which are so deeply stained after the same treatment in the normal adult, are in these young animals in a great measure excluded from participation in the storage of the dye. The bones, if dissected out and cleared, show distinct epiphyses and apophyses sharply outlined within their cartilaginous capsules, and separated by intervening cartilage from the diaphyses. In preparations of this kind, the various centers of osseous development may be readily seen and studied.

The method pursued to attain this end is briefly as follows. Young animals are given a single large dose of a sterile 1% solution of trypan blue. The site of election for the exhibition of the dye is the peritoneal cavity, because the superficial veins are very tiny, and the subcutaneous injection of large amounts of fluid favors pressure necrosis. That the different stages in the process of ossification may be as sharply cut as possible, it is best to administer a large quantity of trypan blue solution at once, rather than to give the same amount of dye in divided doses. Concentrated solutions should be given to avoid the mechanical difficulties involved in the administration of large quantities of fluid. The animal is sacrificed forty-eight hours after staining and the tissues are fixed by injection of a 10% solution of neutral formalin through the blood vessels, followed by immersion in the same solution for a period of from twenty-four to forty-eight hours. The bones are then washed thoroughly, and hardened in ascending grades of alcohol, after which the soft parts are dissected away. The bones are now dehydrated in absolute alcohol, and, after immersion in benzol for twenty-four hours, are cleared in oil of wintergreen after the method of Spalteholz. Oil of wintergreen is an excellent clearing agent and causes little or no shrinkage in the tissues even after long immersion in the fluid. These preparations are conveniently studied with the aid of the dissecting or binocular microscope.

Bones which have been decalcified previous to clearing may be used for study in the same way, and afterward embedded in paraffin directly from oil of wintergreen and sectioned; or bones previously cleared without decalcification may be returned through absolute to 95% alcohol, decalcified and redehydrated, embedded, and cut.

In bones which have been decalcified before clearing, the osteogenetic foci are as distinct though not as opaque as in bones which have not been treated with acid. The loss in opacity is due to the extraction of a small amount of stain which goes hand in hand with the solution of the calcium salts. It is sometimes of advantage in these specimens to introduce as a contrast a very light eosin stain.

The pictures seen in examining these bones are very beautiful. The epiphyseal and apophyseal centers, colored deep blue, are seen embedded in their transparent cartilaginous matrices, separated from the growing end of the diaphysis by a disk of modified cartilage which is readily recognized by the striated appearance which is given it by the arrangement of its cells in parallel columns. The growing ends of the diaphysis are much more darkly stained than the remainder of the bone's shaft; this phenomenon can be readily seen in the advancing ventral ends of the ribs and in the ends of the digital diaphyses adjacent to the epiphyses.

It may be well to emphasize here the fact that stained areas in these bones are not necessarily the so-called primary ossification centers. The stain is deposited in the sites, where, at the time of the exhibition of the dye, active ossification is in progress, and these loci of osteoblastic activity may or may not be identical with the ossification center of the bone or epiphysis under observation. Nowhere is the difference between the primary ossification center and the focus of active growth brought so sharply to the attention of the observer as in the membrane bones of the skull. If one of these bones is examined, it is apparent even to the unaided eye that the vital stain is deposited in a heavy line along the rapidly growing edge of the bone, while the primary ossification centers are seen to be almost entirely without color

The unique feature of this method is that it demonstrates clearly the different ossification areas even before the process of deposition of calcium salts has occurred in any marked degree. In centers where, by the older methods, the new bone is almost or quite invisible (on account of the slight amount of calcium salt present) this method brings out the locus of ossific activity very distinctly.

A large field for the application of this method is suggested by the appearance of the bones which have been examined. In the skull, for instance, graphic demonstrations of the growth of membrane bones and closure of the sutures are afforded. In the base of the skull the various anlagen are easily separated through the sharpness of the contrast between the stained new bone and the intervening masses of cartilage. In the long bones, the epiphyses and apophyses stand out with diagrammatic clearness."

HISTOLOGIC METHODS

Material for histologic study is ordinarily decalcified because of difficulty in cutting undecalcified material. When the process of calcification is not the main interest, the following methods are recommended.

Decalcification. For general histology, the following method has worked well in our experience. Rat bones are fixed in 10% formalin at least 48 hours. They are then transferred to 95% alcohol for at least 24 hours and decalcified in 7% commercial nitric acid for about five days. When delicate tissues associated with bone should be preserved, 1% phloroglucin is added to the nitric acid. The material is washed for 24 hours in running tap water and then soaked in a 5% solution of sodium sulphate in water to remove any traces of acid. Dehydration follows, at least 72 hours each in 70%, 80%, 95% and absolute alcohol. Imbedding is in celloidin or nitrocellulose. For fetal bones, trichloracetic acid (3 to 5%) can be substituted for the nitric acid with no phloroglucin, and the period of decalcification is about three days.

The consistency of the bone can be tested, after three days, with a sharp needle. If the bone can be penetrated even with a little resistance, it can be sectioned satisfactorily. Decalcification can also be accomplished by injections of parathyroid extract (see Burrows[18]) when it is desirable to preserve bone cells or other tissues which might be damaged by too much decalcification. Sections should be cut about 15 microns thick to show bone cells most clearly, including their processes.

Staining. Sections are stained in Ehrlich's acid hematoxylin for five minutes and washed in running tap water for thirty minutes. They are then counterstained in 1% acid fuchsin for fifteen minutes and washed not more than five minutes in distilled water, which also accomplishes differentiation. Dehydration is through 70, 80 and 95% alcohol, 15 minutes each, to beechwood creosote two or more hours for clearing, no absolute alcohol being used.

Other stains that have been used for sections of bone, especially of developing bone, are hematoxylin (Delafield's or Harris') and eosin, (also with azur) iron hematoxylin, alum-cochineal, Calleia's indigo carmine, and Mallory-azan.

For Study of Calcification

When the process of calcification is to be studied, decalcification is objectionable, because such preparations do not give complete pictures of the distribution of bone salts. For criticisms of decalcification when calcification is to be studied, see Dibbelt,[19] Cameron,[8] McLean and Bloom,[20] and Pommer.[21] The use of undecalcified sections of bone is necessary for the autoradiographic demonstration of "bone-seeking" radioactive isotopes.

The following method was used by Shipley, Park, McCollum, Simmonds and Parsons[22] in a study of experimental rickets. Bones were split longitudinally and fixed in 10% formalin. They were then sectioned with a freezing microtome without decalcification, stained with silver nitrate according to the method of von Kossa,[23] and counterstained with hematoxylin and eosin. Blocks of tissue from the ends of the bones used were embedded in parlodion, in some instances without decalcification, in others after short decalcification in Müller's fluid, and stained according to the silver nitrate, hematoxylin and eosin methods just mentioned.

According to McLean and Bloom,[20] it is very important to infiltrate the tissue thoroughly with nitrocellulose, which must then be allowed to become very hard. They used a *C* microtome knife, and there was frequent honing. They employed the method of von Kossa,[23] with modifications. Fixation was in 10% formalin which had been neutralized with $MgCO_3$ and kept in contact with the solid salt until just before use. After rinsing in distilled water, the bones were placed in 75% alcohol (also neutralized with $MgCO_3$) for 24 hours, then in neutralized 95% alcohol for 24 hours, absolute alcohol for 24 hours, and ether alcohol for 24 hours, changing the solution once. The sections were mounted by the Rubaschkin oil of cloves method. After removal of the nitrocellulose by alcohol ether, the slides were transferred through neutralized 75% alcohol to distilled water for 5 minutes. They were then placed in a 2% aqueous $AgNO_3$ solution in the dark for 1 hour and then washed in two changes of distilled water in the dark for ½ hour. Exposure to direct sunlight in distilled water for 1 hour followed. Counterstaining was done with hematoxylin and eosin followed by mounting in damar.

Altmann-Gersh[24] Method. The left hind limb was immersed up to the groin in iso-pentane chilled to —150° to —160° C. When frozen, the limb was broken above the knee and the animal killed. The skin, some muscle and the distal position of the foot were removed without thawing. The remaining portion was transferred to a dehydration chamber and dried at —25° C. It was then immersed for 48 hours in absolute alcohol at the same temperature and then allowed to come to room temperature. Embedding in nitrocellulose, sectioning and staining followed.

Glycerin Mounts. A method which provides sections having more histologic detail was used by Ruth.[25] Decalcification is in 5% HCl. It is considered complete when no precipitate is formed on adding 10 cc. of the solution covering the specimens to an equal volume of equal parts of 5% of ammonium hydroxide and 5% of ammonium oxalate. The tissue is washed in tap water until the acid is removed. It is then placed in a solution of

KOH, 3 gr., glycerin, 20 cc., and water 77 cc., until the matrix becomes semitransparent. It is transferred to rising grades of glycerin (50, 75, and 100%) until completely cleared. The glycerin is removed from the tissue by washing in several changes of 70% ethyl alcohol. Two or three baths of n-butyl alcohol follow to remove the ethyl alcohol. The last butyl alcohol is saved for dissolving paraffin out of sections.

Paraffin infiltration involves at least three baths (one hour or longer in each), according to size of specimen. Sections are cut from 40 to 200 microns thick. The best pictures of lamellae are obtained with sections from 80 to 120 microns thick. The sections are put in a container with enough butyl alcohol to cover them. Two or more baths in butyl alcohol are necessary to remove all traces of paraffin, and this is done in a paraffin oven. Glycerin is added to the butyl alcohol of the final bath in rising percentages, 25%, 50%, and 100%. The sections are mounted in glycerin jelly, and it is better to ring the slides. The composition of the jelly and other details can be obtained from Ruth's article.

BONE WOUND STUDY TECHNIC

The following methods were used by Macklin[26] in a study of the development and function of macrophages in the repair of experimental bone wounds.

"The operations were conducted as follows: The animal having been anesthetized with ether, the top of the head was carefully shaved and sterilized; a median incision was then made and the skin reflected over the parietal areas. The parietal bone having been laid bare, a small trephine of 5 mm. diameter was used to perforate it. In some cases the piece was removed altogether; in others it was replaced, sometimes upside down; and in still other cases a piece of living or dead bone from another rat, or even dead bone from an animal of a different species, was inserted. As a rule two areas were trephined, one in each parietal bone. The wounds were carefully sutured with sterile silk, and the most rigid asepsis was observed. In the same animals, before recovery from the anesthetic, the tibia and femur of the left hind leg and a few of the ribs on the left side were fractured. No attempt was made to splint the bones, so that healing took place under the same conditions as in natural life.

There were slight variations from this general type experiment; in some animals only trephining was done and in others only fracturing. In all, 20 animals were used, which furnished 16 trephined skulls, 15 fractured tibiae, 15 fractured femora, and 12 sets of fractured ribs."

"The animals were killed at various periods during the repair process, the specimens ranging from the second to seventy-first days of healing. The vital-staining technic is the same as that used by Shipley and Macklin"[17] in their work on developing bone, and described in this chapter. "Shortly before the time selected for killing, trypan-blue was administered intraperitoneally in the form of a sterile 1% aqueous solution. As a rule, the dyestuff was given 48 hours before killing and repeated after 24 hours, so that the macrophages were exposed to its action for 2 days. Occasionally the period of exposure was as short as 1 or as long as 3 days. No ill effects followed the exhibition of the dye.

At the time selected the animals were anesthetized and bled, the required tissues being at once dissected out and fixed in 10% formalin neutralized with magnesium carbonate. Care was taken to leave the soft parts surrounding the bones undisturbed. After fixing for 24 hours and washing in running tap-water for same length of time the tissues were passed through a graded series of alcohols ending with two changes of absolute. They were then cleared in benzine and oil of wintergreen, after the method of Spalteholz, and examined. Of the cleared specimens the ribs and skulls were most satisfactory, for it was impossible to make the larger bones transparent owing to their greater density and thickness.

Certain of the skulls and long bones were then decalcified, embedded, and sectioned (Table 1), so that the various stages could be studied microscopically. A few were cleared after having been decalcified; some of these also were afterwards sectioned. The most valuable sections were those simply cleared and mounted with no stain other than the trypan-blue and those lightly counterstained with carmine, which afforded a satisfactory contrast with the blue. Hematoxylin and eosin for cellular detail were used, and also (in special cases) methyl-green and safranin."

CHEMICAL METHODS

Calcium Content Determination. The calcium content in bones was determined by Morgulis and Gies[27] using the following methods.

"After free exposure to the air, for prompt desiccation at room temperature, the teeth and bones were separately crushed and pulverized in a deep porcelain mortar. When, for any reason, we were unable to obtain all the teeth with both the femurs and the tibias from an animal, we used these materials from one side only, and proceeded on a 50% basis, in this way preventing any analytic irregularities that might have resulted from the use of masses that were not anatomically equivalent in kind and proportion.

After the pulverized material had been uniformly mixed and dried to constant weight at 100° to 110°C, an accurately weighed portion of it, practically all the material in each case, was decomposed in a large Kjeldahl digestion flask of Jena glass, with a mixture of equal parts of concentrated sulphuric and nitric acids (Neumann's Method) (see Thierfelder, H. in Hoppe-Seyler, F.: Handbuch der chemischen Analyse. 8th ed., Berlin, 1909, p. 539.) The clear colorless concentrated acid solution freed from most of the mechanical excess of residual sulphuric acid was then diluted to 500 cc. or one liter and kept in tightly stoppered bottles. Calcium was determined in duplicate by McCrudden's method in 100 cc. portions of each solution prepared in this way."

Calcium and Phosphorus Determination. McCann and Barnett[28] in a study of experimental rickets in rats, used these methods.

"The rat was boiled in distilled water for 5 minutes, then the skeleton was dissected out. The water used for boiling was added to the soft parts. The 'large bones' in the *first series* were skull and bones of the extremities, including the ilia and scapulae but not the paws.

The paws were ashed with the other small bones. In the *second series* the skull was ashed with the small bones. This was done because of the difficulty of cleaning the skull and it was felt that better bone figures could be obtained from the other bones alone. To the tissues (evaporated to dryness) Na_2Co_3 was added to prevent possible loss of phosphorus and to give an ash more easily handled. This was not done to the bones which in themselves have an excess of bases.

The parts were ashed in platinum crucibles, the ash was then dissolved in concentrated HCl on the water bath, diluted, and heated for 1 to 3 hours. The phosphorus was determined by titration of ammonium phosphomolybdate. The calcium was determined by McCrudden's method gravimetrically."

Another method for obtaining ash in calcium and phosphorus determinations is given by Bethke, Steenbock, and Nelson[29] (1923).

"The rats were killed by means of ether and the two femurs and humeri dissected out from the fresh tissue. They were dried at 96°C. for 2 days, scraped free from adhering tissues, crushed between the jaws of a large pair of pliers, and quantitatively transferred to an extraction thimble for ether-alcohol extraction. This extraction is absolutely necessary previous to the ashing as the bones of animals vary so much in lipoidal and fat content even when taken from animals on the same ration that the accuracy of percentage values is seriously interfered with if omitted. The extraction was carried out in a Caldwell extractor first with hot 95% alcohol for 18 hours and then U. S. P. 1910 ether, which contained about 2% alcohol, for another 18 hours. After the extraction the bone residue was dried at 96° for 1 hour, carefully weighed, and then incinerated in an electric muffle furnace for 3 hours. The percentage of ash was calculated on the alcohol-ether-extracted basis."

Phosphatase Determination. A microchemical method has been described by Gomori.[30, 31] Smith and Lantz[32] described a method for determining the effect of fluorine upon the phosphatase content.

Calcification Technics. Calcification of hypertrophic cartilage in vitro was studied by Robison and Rosenheim,[33] who described their methods.

Significance of Hexosephosphoric Esters in Ossification. A technic involving the use of silver nitrate staining is given by Robison, MacLeod and Rosenheim.[34] This involves the use of a calcifying solution.

Radioactive Isotopes. Method of Manly and Bale.[35] Radioactive $Na_2\ HPO_4$ was prepared by dissolving phosphorus containing the radioactive isotope in aqua regia and adding Na_2CO_3 until the solution gave a *p*H of approximately 7, "corresponding to Na_2HPO_4." The average daily phosphorus intake was 120 mg. for each rat, and the animals weighed from 110 to 155 grams, except for 2 of 200 grams. In addition, each rat was given by stomach tube a single dose of Na_2HPO_4 prepared as above and equivalent to 4 to 9 mg. of total phosphorus. A single dose gave, before administration, 40,000 to 75,000 counts per minute on one scale of four Geiger-Müller counters. The bones were extracted in boiling 3% KOH in ethylene glycol for 1½ hours and then washed in boiling distilled water, dried at 105°, cooled in a desiccator, and weighed for a study of metabolism of inorganic phosphorus in bones.

CORROSION PREPARATIONS

Corrosion preparations which show Haversian canals and bone cells distinctly have been made by Tehver.[36] The methods described should be adaptable to rat bones. Preparations for microscopic study are obtained. The reader is referred to the original article for details and a figure of the apparatus used.

BIOLOGICAL TEST FOR CALCIUM DEPOSITING SUBSTANCES

A delicate biological test for calcium depositing substances has been described by McCollum, Simmonds, Shipley and Park.[37]

"A group of young rats is placed on diet 3143 for 35-45 days, or until they begin to lose control of their hind legs. They are then divided into two groups, a control group which continues to receive diet 3143 unchanged, and a test group, which is given for the number of days deemed necessary the faulty diet plus the substance which is to be tested.

"When a sufficient number of days have elapsed for the test substance to have produced its effects, the animals are killed and the bones which are to be studied are split longitudinally. The proximal end of the tibia is best for the purpose. One half of the bone is immersed in a dilute silver nitrate solution and exposed to light. It is then examined in the solution through a binocular microscope for the presence of a newly formed line of calcification in the proliferative cartilage. This line of calcium which looks like the cross section of a honeycomb under a strong magnification, is blackened by exposure to light. If this line is present, the test is positive. The line may be visible to the unaided eye in untreated bones. The results of the examination of the gross specimen should be confirmed by study of celloidin or frozen sections from the other half of the bone or other bones. Control rats and rats which fail to give the test do not show the line of calcification. The new line of calcification may extend completely across the bone or may be incomplete or fragmentary, according to the extent of the deposition of the lime salts induced by the substance which is under examination. Since complete starvation also causes the typical linear deposit of lime salts to appear in the cartilage of rachitic animals, the food intake of both test and control rats must be carefully watched."

MEASUREMENTS OF BONE GROWTH

Schour[38] made multiple intraperitoneal injections of 0.5 cc. of 2% alizarin red S into growing rats. He then measured in microns the distance between two red rings found in alveolar bone and divided this amount by the number of days intervening between the injections.

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19

The Eye of the Albino Rat

By

W. E. FRY

THE fundus examination of the albino rat is similar to the fundus examination of any other laboratory animal. By preference the examination should be conducted in a dark room, from which all exterior light is excluded. An electric ophthalmoscope with a means of concentrating the light should be used.

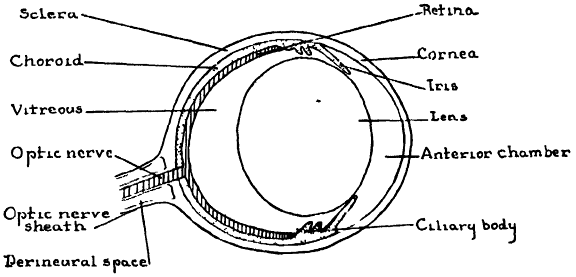

Fig. 171. Diagrammatic cross section of rat's eye. Note the shallow anterior chamber and the relatively large size of the lens. Two ciliary processes appear to be fairly constant.

The pupil must be dilated by atropine. Dilatation is necessary for two reasons and takes place rapidly and fully. The cornea of the rat frequently contains irregularities and opacities which make examination with a small pupil difficult, and in addition the examiner, with ophthalmoscope, cannot approach close to the eye because of the necessity of avoiding the sharp teeth of the unanesthetized animal. Anesthesia is not required. The animal may be held sufficiently quiet in the hands of an assistant wearing heavy gloves.

The media of the eye cannot be satisfactorily examined. There are always corneal opacities that prevent a detailed scrutiny of the lens and vitreous. Most of the animals are highly hyperopic—about five diopters. The lens on section is nearly spherical and occupies approximately two-thirds of the intraocular cavity (see Fig. 171). The fundus yields a bright pinkish-white reflex. Figure 172 is the drawing of a normal fundus. There is no disc and no macula, and there are no medullated nerve fibers. The position of the disc is recognized as the point where the retinal vessels emerge, and at times is marked by a small irregular, quadrilateral, whitish area. The vessels extend in fairly straight radial lines from this whitish area. There are few branches. As in other fundi the veins are slightly broader and darker than the arteries. The only pathologic changes noted have been in broadening of the veins in venous congestios and small linear hemorrhages of the nerve-fiber layer of the retina near the position of the disk. (See Fig. 173.) Such fundus changes were seen in the albino rat after an actively growing cerebellar tumor had been successfully transplanted. These changes were verified by the use of the slit lamp and by histologic sections.

Fig. 172

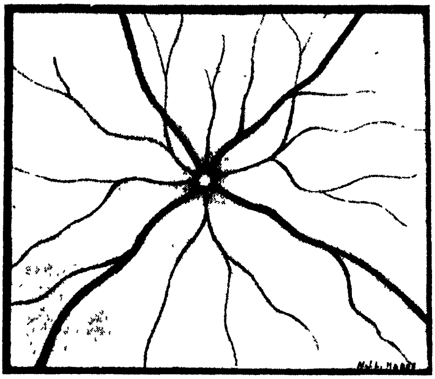

Fig. 173

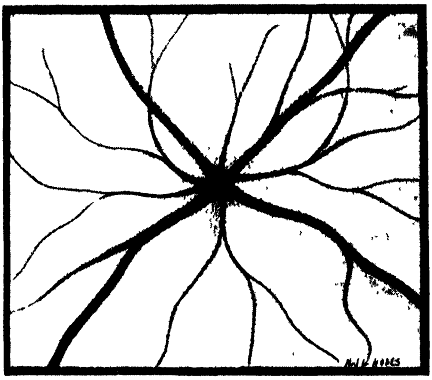

Fig. 172 (left). Drawing of the fundus of a normal albino rat.

Fig. 173 (right). Drawing of the fundus of an albino rat bearing an actively growing cerebellar tumor. Note the venous engorgement and the appearance of fulness in the region of the disc.

The slit lamp is used in the following manner. The eye of the rat is enucleated and fixed for 24 hours in formalin. Then the cornea is cut away at the limbus and the lens removed. The posterior two-thirds of the eye thus obtained is mounted in a bottle in 70% alcohol and suspended so that it can be illuminated with the slit lamp and examined with the corneal microscope. In the normal rat eye thin thread-like lines of vessels are seen converging at a point which corresponds to the optic disc. No disc outline is distinguishable. In eyes showing pathologic changes over-filled veins are seen. No mushroom-like disc swelling is even seen.

For the histologic preparations formalin makes a satisfactory fixative and nitrocellulose an excellent embedding medium. A section through the posterior poles of the eye, shows the optic nerve, enclosed in a thin sheath. The vessel which supplies the retina enters the nerve just behind the sclera, and apparently is a well-developed posterior ciliary vessel. The retina over the position of the entrance of the optic nerve is nearly flat. The veins are not unusually full (see Fig. 174).

In an eye removed from an animal with an actively growing tumor and with ophthalmoscopic changes the histologic changes noted were a marked engorgement of the veins of the nerve head and an edema of the nerve-fiber layer, adjacent to the nerve head (see Fig. 175).

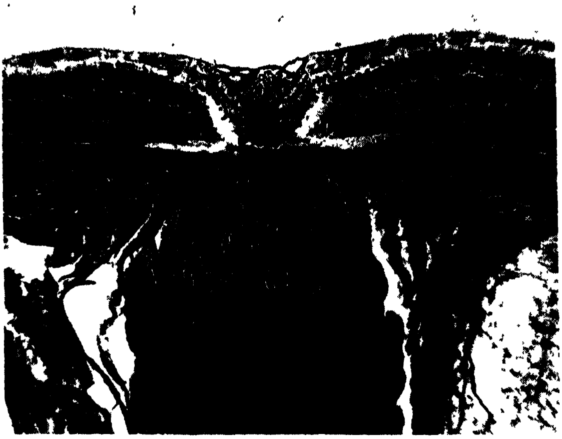

Fig. 174. Section through the optic disc of a normal albino rat.

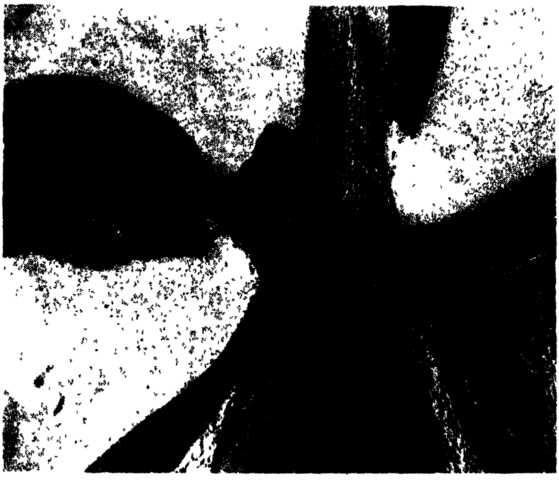

Fig. 175. Section through the disc of an albino rat bearing an actively growing cerebellar tumor. Note the venous engorgement and the edema of the retina about the disc. The retinal detachment is an artifact.

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20

Protozoan Parasites of the Rat

By

D. H. WENRICH

INTRODUCTION

THE rat has proven to be a very convenient animal for the study of parasitic protozoa since it harbors a considerable number of protozoa which are natural parasites and is experimentally susceptible to many others.

In this section attention will be given primarily to the parasitic protozoa of the Norway rat, although it will be convenient in certain cases to discuss those of closely related species. Illustrations or partial descriptions are given for identification. The discussion deals with the occurrence of the parasites, some of the experimental work done upon them, upon the rat as an experimental host, and upon the technics employed in the study of the parasites and their relation to the host. No effort has been made to secure completeness in the discussions or references to literature. The references are mostly the more recent ones or those from which earlier references can be obtained.

THE PROTOZOA OF THE DIGESTIVE TRACT

Parasites Living in the Lumen

Parasites of the Small Intestine. In the small intestine, *Giardia muris* (Fig. 176, 1 and 2) and *Hexamita muris* (4)* are commonly found. Less frequently one finds *Giardia simoni* (3) which seems to be morphologically identical with *G. lamblia* from the small intestine of man.[51]

Parasites of the Cecum. *Flagellates.* In the cecum may be found *Hexamita pulcher* (5), *Trichomonas muris* (6), *T. minuta* (7), *T. hominis* (8), *T. wenyoni* (9) (called *T. parva* by Wenrich[68]), *Hexamastix muris* (10) (found by Wenrich[67] only in the black rat, *Rattus rattus*), and *Chilomastix bettencourti* (11 and 12). Occasionally some others apparently identical to those from man are found. For example, an incidence of 1.7% of Retortamonas (Embadomonas) was reported for 2515 wild rats caught in Baltimore[2] and the writer has recently found an Enteromonas-like flagellate (13) in a wild Norway rat caught in Philadelphia.

Amebae. *Entamoeba muris* (14 and 15), which closely resembles *E. coli* from the human colon, including the production of mature cysts with eight

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* The numbers in parentheses refer to Figures 176 and 177.

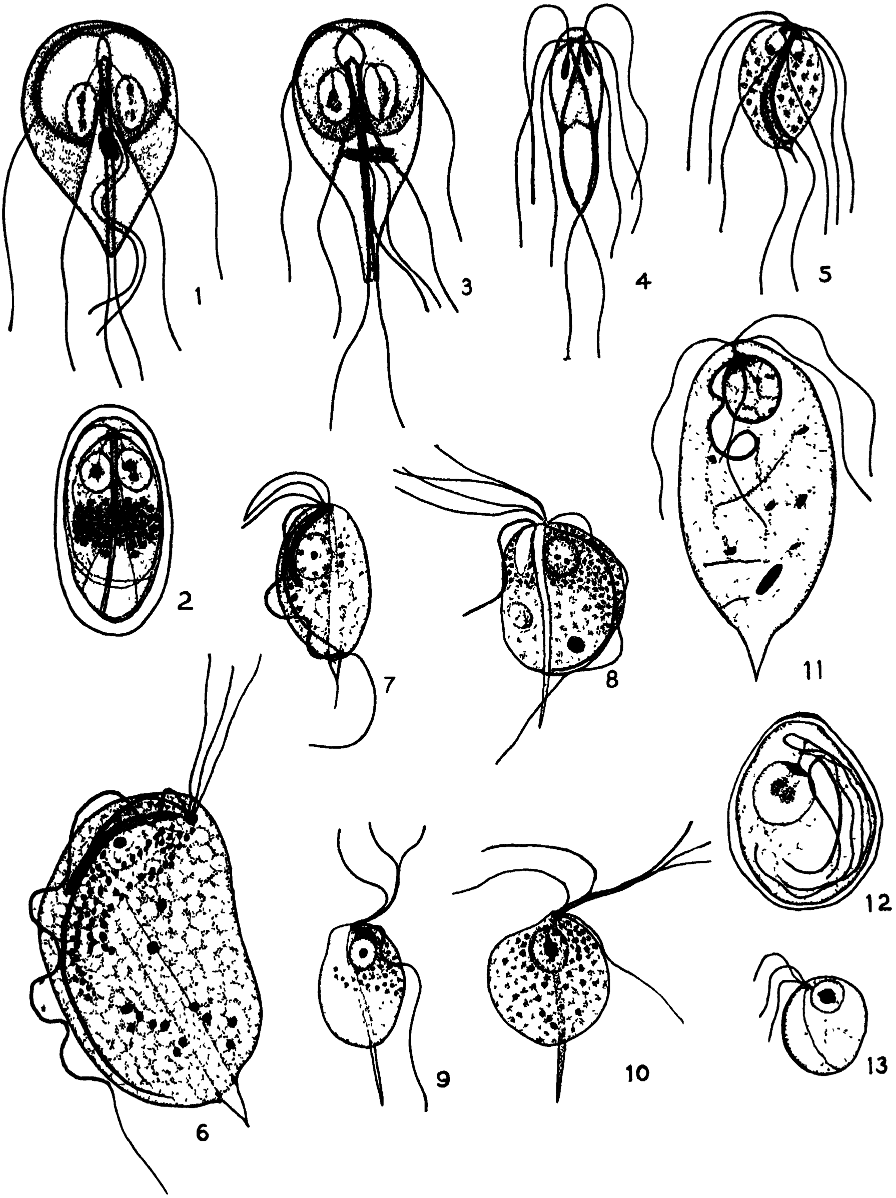

Fig. 176. Intestinal Flagellates. All Approximately × 3000.

1. Trophic stage of *Giardia muris*
2. Cyst stage of *Giardia muris*
3. Trophic stage of *Giardia simoni*
4. Trophic stage of *Hexamita muris*
5 Trophic stage of *Hexamita pulcher*
6. Trophic stage of *Trichomonas muris*
7. Trophic stage of *Trichomonas minuta*
8. Trophic stage of *Trichomonas hominis*
9. Trophic stage of *Trichomonas wenyoni*
10 Trophic stage of *Hexamastix muris*
11 Trophic stage of *Chilomastix bettencourti*
12 Cyst stage of *Chilomastix bettencourti*
13. Trophic stage of Enteromonas-like flagellate

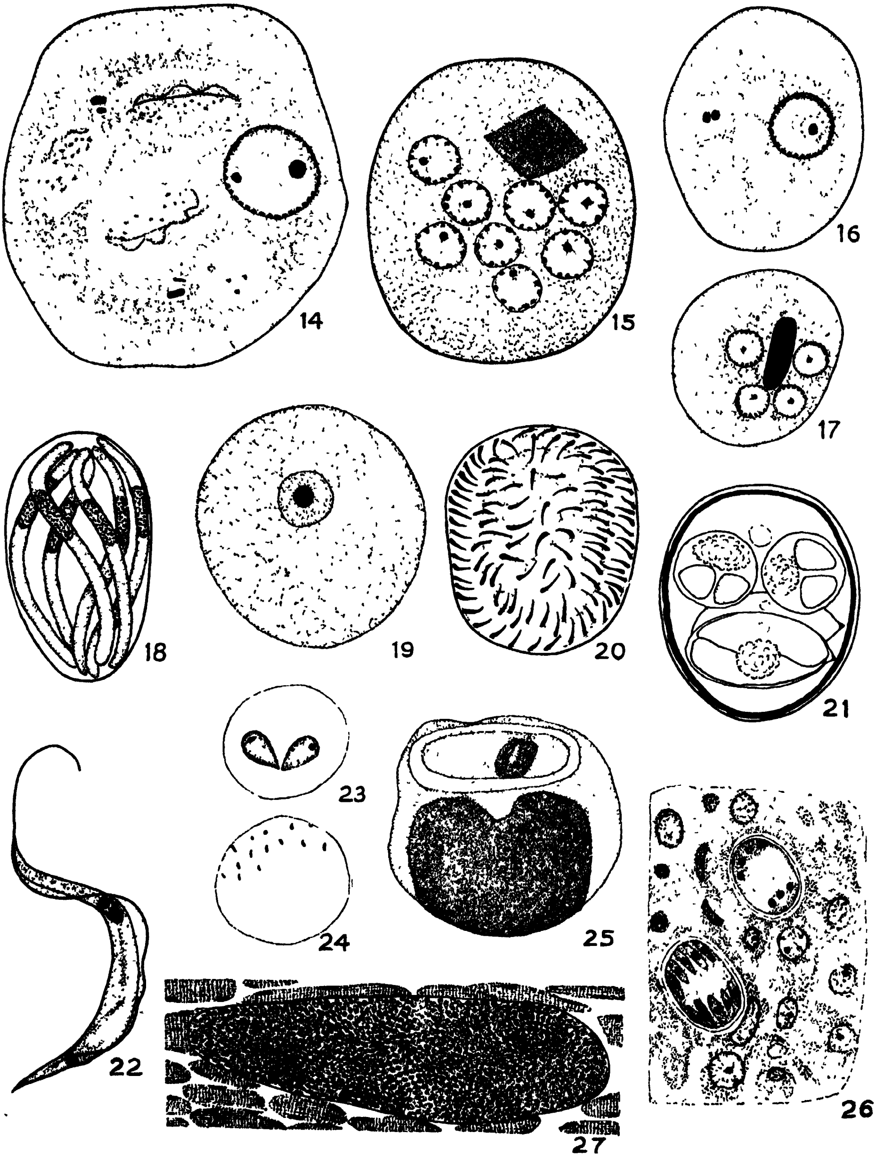

Fig. 177. Intestinal Parasites. 14 to 21, ×2000; Figs. 22 to 25, ×2750; Fig. 26, ×400; Fig. 27, ×200. 14 to 17 after Wenrich;[69] Fig. 18 from slide loaned by R. L. Roudabush; Fig. 21 after Becker, Hall and Hager;[8] Fig. 23 after Fantham;[21] Figs. 25 and 26 after Miller.[47]

14. Trophic stage of *Endamoeba muris*
15 Mature cyst of *Endamoeba muris*
16. Trophic stage of *Endamoeba histolytica* from a rat
17 Mature cyst of *E. histolytica* from a rat
18 Group of merozoites of *Eimeria Nieschulzi*
19. Macrogamete of *Eimeria sp.*
20. Microgametes of *Eimeria sp.*
21 Oocyst of *Eimeria separata*
22 *Trypanosoma lewisi* from blood of rat
23. Rat erythrocyte containing *Babesia muris*
24. Rat erythrocyte containing *Bartonella muris*
25 Rat lymphocyte containing gametocyte of *Hepatozoon muris*
26. Rat liver tissue containing schizonts of *Hepatozoon muris*
27. Oblique section through a "Miescher's Tube" of *Sarcocystis muris* in rat muscle

nuclei (15), is commonly present in the cecum of rats. Other amebae, apparently identical with species found in man occur occasionally, such as *Entamoeba histolytica* (16 and 17), *Endolimax nana,* and *Iodamoeba bütschlii*; and all of these have been experimentally transferred to the cecum of rats (35).

Ciliates. Only one ciliate, *Balantidium coli,* which is very common in the cecum and colon of pigs and rarely becomes a parasite of man, is occasionally found in rats. It has been successfully transferred to rats by various authors.[58, 22]

Host-parasite Relations. The above-mentioned intestinal protozoa are limited primarily to the lumen of the canal although *Entamoeba histolytica* and *Balantidium coli* are sometimes associated with ulcers in the cecal wall. Kofoid and Christiansen,[37] who described binary fission in *Giardia muris,* believed that this flagellate was somewhat pathogenic to its hosts, but Boeck[10] did not share this opinion. The other flagellates are supposed to be nonpathogenic, although the writer has often noted the presence of many leukocytes in the contents of the cecum when this organ contained a heavy infection with *Trichomonas hominis.*

The lumen dwellers apparently nourish themselves with the food with which they are surrounded and they multiply by binary fission. All except the trichomonads are able to assume a state of inactivity surrounded by a protective membrane and in this encysted condition they pass out with the feces and are transmitted to new hosts when they contaminate the food or drink of other individuals. The trichomonads do not seem to produce completely formed cysts although cyst-like stages are not uncommon. Experiments have shown that the trichomonads can pass through the stomach and into the intestines in a viable state without encystment.[26, 70]

Methods. *Obtaining Fresh Material.* Fecal pellets will frequently contain the cysts of those intestinal protozoa capable of encystment. Since cyst-formation occurs irregularly or sometimes cyclically, many daily examinations of feces may be necessary before a determination of the intestinal protozoa can be reasonably complete. Handling rats or stroking the back may induce the deposition of fresh pellets. Pellets may also be squeezed through the anus by gentle abdominal massage. Soft feces containing trophic stages from the cecum may be obtained by feeding sunflower seeds[25] or other mild laxative. Purging may be effected by feeding stale bread soaked in a saturated solution of magnesium sulfate.[34] Fresh material may be obtained by killing the rat and opening the abdominal cavity and making incisions in those parts of the digestive tract to be investigated. If it is desired to keep the rats alive, material may be obtained by surgery under ether anesthesia.

Examination. For the recognition and study of living protozoa from the intestinal tract, a small bit of feces or intestinal or cecal contents is stirred up in physiologic salt solution on a micro-slide, covered with a cover glass and examined. For prolonged study the cover should be ringed with petrolatum or a similar substance. Ringer's solution, preferably Drbohlov's[19] modification (6 gm. NaCl, 0.1 gm. KCl, 0.1 gm. CaCl, 0.1 gm. NaHCO$_3$, 1000 cc. distilled H_2O) or 0.75% NaCl may be used. Active flagellates can often be identified by an experienced observer in the living state, especially when studied with the high dry objective. Sluggish flagellates can frequently be revived by raising the temperature to that normal for the host. This may be done by making the study in a warm room, by placing the microscope in a warm cabinet, or by use of a warming stage.

Cultures. The incidence of *Trichomonas hominis* can be determined more accurately by culture than by direct examination of feces. This species will grow on many media but a convenient one is 0.2 to 0.5% of Loeffler's dried blood serum in 0.75% NaCl solution or Ringer's solution. This species can frequently be grown at room temperature. Simple and satisfactory culture media have not been developed for the other intestinal flagellates. Giardia has been especially refractory. *Entamoeba histolytica* from the rat may be cultivated fairly easily since a great many methods have been devised for the culture of this species, e.g., liver infusion agar slants covered with fresh horse serum diluted 1 to 6 with 0.8% NaCl or Ringer's solution.[14] *E. muris* is not readily cultured.

Temporary Killing and Staining. Temporary killing for counting flagella and study of other details, may be accomplished by diluting the feces or intestinal contents with one per cent to two per cent osmic acid, or Lugol's Iodine solution diluted to a cherry red with distilled water. Killing and differential staining may be accomplished in one step by use of Repart and Petit's fluid.

Dried Smears. For flagella counting and for some other details a dilution of intestinal contents or feces in physiologic salt solution may be spread thinly on a slide or cover, then held over the mouth of a bottle containing one or two per cent of osmic acid solution for 10 seconds, then dried as quickly as possible. The preparation is fixed for five to ten minutes in pure methyl alcohol, dried and stained for 30 to 60 minutes with Giemsa's stain, diluting the stock stain with neutralized distilled water in the proportion of one drop of stain to one cc. (20 drops) of the water. The stain is then washed off with water and the preparation dried as quickly as possible, or blotted with smooth lens paper. It may then be mounted in neutral balsam or damar.

Permanent Slides from Wet Smears. Intestinal or cecal contents are usually diluted with physiologic salt solution, smeared thinly on a slide or cover glass and placed in fixing fluid before drying can occur. The most commonly employed fixing fluid is Schaudinn's, made by adding one part of 95% alcohol to two parts of a saturated solution of mercuric chloride in water. Just before using 1 to 5% of glacial acetic acid is added. Many other fixing agents give fairly satisfactory results. For preservation of the parabasal body of flagellates, chrom-acetic or weak Flemming's solution may be used. Heidenhain's iron alum haematoxylin is the most satisfactory stain. The mordanting and staining can be shortened to ten to fifteen minutes for each if the reagents are kept at incubator temperature; otherwise at least 30 minutes should be allowed for each.

Estimation of Numbers. Quantitative results may be obtained in various ways. The number of flagellates in the cecum may be estimated after performing a laparotomy under ether anesthesia. Then 0.2 cc. of cecal material is placed in 1.8 cc. of physiologic salt solution in a short pyrex tube and thoroughly mixed. In counting the flagellates, a drop of this 1 to 10 suspension is placed on a blood-counting slide (Leitz) and the number of animals in four groups of large squares counted. This gives the number of animals in 0.1 cu. mm. of 1 to 10 suspension, and this figure multiplied by 100 gives the number of protozoa per cu. mm. of the cecal contents.[53]

In rats that have been freshly killed, all the contents of the cecum may be squeezed out onto a piece of waxed paper, placing this in the bottom of a Stoll flask which is then filled to the 60 cc. mark with Ringer's solution kept at 37 degrees C. The flask is stoppered, placed in the incubator and shaken several times during the succeeding hour or two. Any remaining lumps are broken up with an applicator. The counts are then made with a haemacytometer in the usual way, and, multiplying the number per cc. of the dilution by 60, the total number in the cecum is obtained.[28]

Protozoa-free Rats. It is often desirable to have rats free from intestinal protozoa especially when experimental inoculations are to be made. Young rats do not usually become infected until about the time of weaning or subsequent thereto. Therefore removing the young rats from the mother at 17 days of age and keeping them free from contamination, will result in a colony of protozoa-free rats.[54]

Entamoeba muris may be eliminated by feeding a diet high in protein content,[29] and trichomonads can be removed with carbarsone.[27, 23] Gabaldon[23] found that 150 mg. or more of the drug per kg. of body weight given orally each day for five days would eliminate the trichomonads. This was given in 1% solution in 0.5% sodium bicarbonate as a substitute for drinking water or introduced directly into the stomach daily for five days with a syringe. A single dose of 1000 mg. per kg. of body weight was also effective given either orally or intravenously.

Starvation for four to seven days greatly reduces the numbers of Giardia in the small intestine of rats or eliminates them entirely, and aldarsone is effective against these flagellates. A treatment consisting of two days starvation, then the administration of 1.5 to 3 cc. (depending on the size of the rats) of a 10 per cent solution of aldarsone by intragastric injection followed by two or more days of starvation is highly effective in eliminating Giardia.[3] Rats will lose their Giardia infections if kept for five months in screen-bottomed cages so that the fecal pellets could drop through, thus preventing reinfections.[30]

The rat has proven to be a convenient animal for the study of various intestinal protozoa, not only those normally found in the rat, but others, such as *Trichomonas hominis, Giardia lambia, Entamoeba histolytica* and *Balantidium coli.*

Parasites of the Intestinal Wall: Coccidia

The Species of Coccidia. Several species of coccidia belonging to the genus Eimeria have been found in rats. The general features of their life histories are similar. The infectious stage is the sporulated oocyst (21) which contains four spores each with two sporozoites. When ingested, oocysts pass to the intestines of the host where the slender sporozoites emerge from their envelopes and penetrate the cells lining the intestinal tract. The parasites round up and grow at the expense of the host cells and, by a process of schizogony, give rise to a number of banana-shaped merozoites (18). These escape from the remnants of the host cell, invade other cells and repeat the process of schizogony. After several generations produced in this manner, the merozoites invade host cells to become gametocytes. Each macrogametocyte produces one macrogamete (19), and each microgametocyte produces many small comma-shaped microgametes (20). A microgamete fertilizes a macrogamete, thus producing a zygote which becomes surrounded by a protective membrane and thereby becomes an oocyst. In most species of Eimeria, especially those of warm-blooded hosts, no further development takes place until the oocysts are deposited outside the host in the feces. Under favorable conditions of moisture and temperature, development within the oocyst leads to the formation of four spores each containing two sporozoites (21). The oocysts are highly resistant to the action of chemicals but are injured or killed by complete desiccation or a temperature of 50°C. or higher for 10 minutes. The characters of the oocysts and their contained spores together with sporulation time are used in generic and species determinations. Useful also are: (1) the prepatent period, between the ingestion of oocysts and the appearance of oocysts in the feces; and (2) the patent period, during which oocysts are discharged. These periods tend to vary from species to species.

Yakimoff and Gousseff[73] recognized eight species of Eimeria from the rat but the validity of some of them has not been confirmed. Fairly complete life histories of three species have been worked out so that these can be fairly accurately characterized. The following data are taken from Roudabush:[57]

E. nieschulzi Dieben (-*E miyairii*[4, 46]); oocysts ovoidal, averaging 22.5 μ x 17.8 μ; oocyst wall smooth or granular; sporulation time 65 to 72 hours, prepatent period 7 to 8 days, patent period 5 to 8 days.

E. separata Becker and Hall; oocysts ellipsoidal, averaging 16.06 μ x 13.85 μ; wall smooth; sporulation time 27 to 36 hours, prepatent period 5 to 6 days, patent period 4 to 6 days.

E. miyairii Ohira (-*E. carinii*[4, 46]); oocysts subspherical to spherical, averaging 24.38 μ x 22.12 μ; oocyst wall thick, rough, radially striated; sporulation time 96 to 120 hours, prepatent period 6 days, patent period 6 to 7 days.

As described by Roudabush[57] *E. nieschulzi* invades the mucous epithelium of the small intestine, cecum, and colon. Intracellular stages of *E. separata* are found in the surface epithelium of the cecum and colon. Even heavy infections do not usually produce clinical symptoms. Endogenous stages of *E. miyairii* occur in the mucous epithelium, usually of the villi of the small intestine.

Becker and his associates[4] have demonstrated that susceptibility to infection with coccidia varies considerably with the strain of rats used. They have also demonstrated what had been shown for other coccidia in other hosts: (1) that there is a high degree of host-parasite specificity, and (2) that rat hosts may be specifically immunized against reinfection by the same species of Eimeria. No immunizing agent was detected in the serum or tissues of immunized rats, nor was immunity induced by injecting coccidian proteins. Neither blocking of the reticulo-endothelial system nor removal of the spleen produced any noticeable effect on experimental infections.[6] Later it was concluded that immunization resulted in preventing sporozoites from penetrating the epithelial cells of the immunized rats.[49]

A rat may be infected with a single oocyst of *E. nieschulzi* (*E. miyairii*)[5] and merozoites from the intestine of one rat may be used to infect another rat.[56] Becker and Morehouse[9] as recorded in a series of papers have attempted to influence development of the endogenous cycle of *E. nieschulzi* (*E. miyairii*) by dietary factors. No significant changes in numbers of oocysts discharged by the host could be produced by such measures as general vitamin and mineral deficiency, vitamin-A deficiency, vitamin-D deficiency, high-casein diet, high-salt diet, low-salt diet, skim-milk supplements, tobacco-dust supplements, and iodine or vinegar in the drinking water. However, when yeast was used as the source of vitamin G, oocyst production was apparently stimulated to the maximum. This factor which apparently stimulates development of *E. nieschulzi* in the rat is called coccidibios.

Methods. *Examination, Sporulation and Measurement.* Fecal examination for the discovery of oocysts may be conducted in the same manner as for other intestinal protozoa (see p. 430). Small numbers might be missed unless concentration methods were employed. Feces can be stirred up in water, passed through 16-mesh wire screens or a single thickness of cheese cloth to remove larger particles, then concentrated by centrifuging or by sedimentation. Flotation in a saturated salt solution will bring the oocysts to the top of the fluid.

Unsporulated oocysts are usually allowed to sporulate by placing feces in 1% chromic acid or 2 to 5% potassium dichromate in a shallow vessel, such as a petri dish. Oxygen is thought to be essential to development.

Measurement of oocysts may be made with an ocular micrometer or with a camera lucida.[8]

Quantitative Determinations. To determine the relation of the size of infective dose to number of oocysts eliminated, and duration of infection and immunity, Hall[24] used inbred rats (Wistar-A strain) and an inbred strain of Eimeria. The latter was obtained by inoculating a rat with a single oocyst, isolated under the microscope with a micropipette and deposited in the throat of a clean rat. When oocysts appeared in the feces, a second infection with a single oocyst was made and the process was repeated for another generation.

For noting the effect of a definite number of oocysts, the oocysts were either isolated under the microscope for numbers up to ten, or estimated by counting dilution samples for higher numbers. Rats on experiment were kept in individual cages made of three-mesh hardware cloth with an aluminum or enamel pan, nine by twelve inches, underneath to catch the fecal pellets. Three hundred cc. of 0.25 to 0.5% solution of "Kreso" were placed in the pans.

To determine total numbers of oocysts from one infection, pellets were allowed to accumulate during the patent period, then broken up with a masher made of a rubber stopper with a glass tube as a handle. The whole contents of the pan were then transferred to a vessel and homogenized with an electric mixer. Water was added to make a measured amount, and the material stirred again. It was then passed through 16- and 24-mesh wire screens to remove coarser particles. After a thorough stirring a small quantity was placed in a haemocytometer and a number of counts made. The total yield could then be readily calculated.

Methods for Assaying Foodstuffs. Methods for making assays of foodstuffs in terms of their effect upon the development of *Eimeria nieschulzi* have been evolved.[7] The Wistar-A strain of rats and an inbred strain of parasite were used. Before the experiment started rats were grown on Steenbock's growing ration. During experiments the following reference diet was used as a control: (by parts) beet sugar, 72; casein (unextracted), 15; salt mixture (Hawk and Oser), 4; lard, 3; cod liver oil, 2; Fleishmann's powdered yeast, 4. Experimental diets were prepared by substituting the material to be tested for the yeast and a part of the sugar. More concentrated materials were fed at a 10% level, and others at a 30% level. For example, the oats diet consisted of: beet sugar, 46; casein, 15; salt mixture, 4; lard, 3; cod liver oil, 2; and ground whole oats, 30. The rats were handled and oocyst counts made in essentially the manner described above. The results were interpreted as supplying a yeast coefficient (F) expressed as "the ratio of the number of oocysts passed by ten or more rats on a test diet to the number passed by ten or more rats on the reference diet."

THE PROTOZOA OF THE BLOOD AND TISSUES

The Trypanosomes. *Trypanosoma lewisi. T. lewisi* represents a genus of blood flagellates the species of which are very numerous and widely distributed through all classes of vertebrates. In most cases trypanosomes are transmitted by blood-sucking invertebrates, in which a part of the life cycle frequently takes place. Two or three species produce disease in man and a number of others are pathogenic to domestic animals in various parts of the world.

The common rats throughout the world are frequently infected with *T. lewisi*, which is transmitted in nature primarily by the rat flea, ceratophyllus fasciatus.[48] This species was reported in 7% of 100 wild rats caught in Washington, D. C.,[52] in 7.4% of 2515 wild rats caught in Baltimore,[2] in 11% of 100 rats caught in St. Louis[66] and in 47.5% of 40 rats caught in Indiana.[13] Similar findings have been reported from various other parts of the world.

Trypanosoma lewisi appears to be specific for the common rat, other rodents having similar but different species. Under certain conditions this species may be transferred to other laboratory animals and recently a human being was reported to be infected by this species.[32]

T. lewisi is said to be nonpathogenic to rats although pathogenic strains have been reported.[11] Taliaferro and his associates[61] have demonstrated the existence of at least two antibodies in rats infected with *T. lewisi*. One of these (ablastin[62]) inhibits reproduction and the other is trypanolytic. Splenectomy is said to have no marked effect on the development of *T. lewisi* in rats[59] and ablastin can be transferred as effectively to rats that have been splenectomized and intensively blocked with India ink as to normal rats but such passive immunity lasts only a few days.[63] Passive transfer of the trypanocidal antibody is slightly but definitely impaired in splenectomized and blocked animals as compared to normal rats. The passive immunity in this case lasts only a few days and then small numbers of surviving flagellates may multiply because ablastin fails to develop. In normal rats such survivors would be prevented from multiplication by a developing ablastic immunity.[63]

T. lewisi is pathogenic to young rats up to 25 days or more of age.[31, 20] Older rats develop an adequate defense.[20] Immunity may be transferred to nursing rats through the mother's milk whether the mother has an active acquired immunity[16] or a passive immunity.[18] Younger rats, 20 days old or less, may be protected by administering antiserum per os. Rats over 20 days of age cannot be protected in this manner.[17]

Trypanosoma cruzi. Recent studies[72] have shown that *T. cruzi,* cause of Chaga's disease in man in South and Central America, occurs naturally in southern California in the wood rat, *Neotoma fuscipes macrotis,* and in the blood-sucking bug, *Triatoma protracta,* which serves as transmitting agent. This trypanosome had earlier been described from the bug.[38] It has been transferred to albino rats, albino mice, rhesus monkeys, a puppy, an opossum, two species of wood rat (Neotoma) and five species of white-footed mice (Peromyscus).[72]

Trypanosoma cruzi is a tissue-invader, penetrating the cells and tissues outside the blood stream, especially the heart and other muscles. These intracellular flagellates round up and become aflagellated leishmanoid forms. They multiply extensively forming masses of small rounded aflagellate stages. These resume the flagellated condition, re-enter the blood stream and are thus carried to other organs and tissues.

Studies on *T. cruzi*[40] have shown that, as in *T. lewisi*, young rats up to 20 days of age are much more susceptible than older rats, the resistance rising with age to a maximum at full maturity. Antiserum from comparatively resistant adult rats confers no protection to younger rats. The age difference in susceptibility applies to infection per os as well as to intraperitoneal infection.[42] It is also noted that intensity of infection reflects a seasonal variation, the summer months being correlated with lower intensities as compared with the winter months.[41]

Although rats are said not to become infected by eating the flesh of other rats containing *T. rhodesiense*,[15] culture forms of *T. lewisi* may infect rats when introduced into the mouth, while blood forms rarely do so.[33] Young rats up to 20 days of age may be infected with an infective suspension of *T. cruzi* dropped into the mouth, or up to 30 days of age when the suspension is injected into the stomach.[42]

Methods. Examination of blood for trypanosomes is usually carried out by snipping off the tip of the tail with scissors and securing a drop of blood on a slide, placing a cover-glass over it and examining under a microscope. The active movements of the flagellates enable one to detect their presence very easily. Dilution of the blood with physiologic salt solution, Ringer's solution or citrate solution (0.5 Gm. sodium chloride and 0.5 Gm. sodium citrate in 100 cc. of distilled water) will enable one to study the individual flagellates more readily. In the case of *T. cruzi*, the relatively small numbers in the circulating blood are not a safe criterion as to the total numbers of parasites, since large numbers may be present in the tissues.

Stained preparations are made by making thin dried blood smears and staining them with one of the common blood stains such as Giemsa's, Wright's or Leishman's.

Inoculations are usually made by diluting the blood containing the trypanosomes with citrate solution, Ringer's solution, or physiologic salt solution, and injecting this intraperitoneally with a syringe and hypodermic needle. For intragastric injection of trypanosomes into young rats, one may employ a syringe with a 22-gauge hypodermic needle with the bevelled tip filed off and the edges smoothed. In older rats a catheter can be used on partly anesthetized animals.[42]

As a means of sending *Trypanosoma cruzi* from one laboratory to another, cardiac or splenic tissues from a heavily infected rat can be placed in Wassermann tubes each containing two cc. of buffered Ringer's or Locke's solution and the tubes sealed. Tests show that the parasites retain their viability for ten to fifteen days at either room or refrigerator temperature. Tissues can be taken out, ground up without abrasives in one cc. of fresh solution and the fluid injected into a rat.[39]

Hepatozoon Muris. *Hepatozoon muris* is a parasite which develops in the liver cells and leukocytes of rats and has other developmental stages in the transmitting arthropod host. The life history as worked out by Miller[47] involved the mite, *Lelaps echidninus*, as the transmitter. When a rat swallows a mite containing spores, sporozoites are released in the small intestine. These penetrate through the intestinal wall into the blood stream and are carried to the liver. Here they invade the liver cells and undergo schizogony (Figs. 177, 26). Merozoites so produced may repeat the cycle in other liver cells or may enter the blood stream to be taken up by the large mononuclear lymphocytes in which they become encysted (25). When a mite ingests blood containing these encysted gametocytes, the latter are released in the stomach of the mite. They become gametes, fuse in pairs to produce zygotes which penetrate the stomach wall and become encysted as oocysts in the tissues of the mite. Each oocyst gives rise to 50 to 100 spores, each spore containing about 16 sporozoites. The mite is then in a condition to infect another rat. According to Miller, experimentally infected rats may die from the infection. Closely related species have been found to be transmitted by other bloodsucking arthropods such as lice, fleas, and ticks. Lists of species and hosts of Hepatozoon in mammals have been published.[71, 50] Infections have been reported in rats in England, Portugal, Panama, Brazil, Australia, and various parts of Africa. Only one infection was reported among 2515 wild rats caught in Baltimore,[2] but 17 out of 100 rats caught in Washington, D. C., were found to be infected.[52]

The gametocytes of this species would be detected in blood smears stained by one of the regular blood stains.

Babesia Muris. Parasites of this group are wide-spread agents of disease among domesticated animals and are usually transmitted by ticks. Fantham[21] described, under the name *Piroplasma muris*, a parasite in the red-blood cells of white rats in London which appears to belong to the genus Babesia (23). A parasite of this type was reported from *Mus decumanus* (*Rattus norwegicus*) in West Africa.[45] Similar parasites have been reported from other small rodents as well as from a wide range of other mammalian hosts.[71]

These parasites can be detected in thin blood smears stained with Wright's or other blood stain.

Other Blood Parasites. Rats are subject to an anemia, especially marked after splenectomy, associated with the presence of small coccoid or bacilloid bodies on or in the red blood cells (24). These bodies are commonly referred to as *Bartonella muris*. Similar bodies are widely distributed among mammals, including man, and several names have been applied to them, including Grahamella and Eperythrozoon. According to Brumpt[12] Grahamella and Bartonella are synonymous, Grahamella having priority, but Lwoff[44] regards them as distinct and most recent authors have kept the names separate.

Mice may acquire an immunity to Bartonella reinfection which depends largely on the spleen.[1] A strain of Bartonella, virulent for mice was found to be non-virulent for rats.[1] Anemia may be produced in non-splenectomized rats by blockading the reticulo-endothelial system with India ink.[55]

Bartonella and related bodies are apparently world-wide in distribution among rats and mice as well as among many other animals.[36] They appear as deeply stained bodies on or in the erythrocytes in blood smears stained in the usual manner. Bartonella infections have been controlled with neosalvarsan, 0.014 Gm. per 100 Gm. of rat, given intramuscularly.[20]

Sarcocystis Muris. This parasite occurs as elongated white cylinders called "Miescher's tubes" (27) with rounded or tapering ends in the striated muscles of rats and mice. While earlier stages in the muscles are only 25 microns in length, later stages may attain a length of a few centimeters and be distinctly visible to the unaided eye in fresh muscle. When sections of infected muscle are examined with the microscope the tubes are seen to be divided by very thin septa into compartments and these are filled with crescent or banana-shaped spores about 10-15 microns in length.

Rats and mice can be infected by feeding them with muscle containing spores or with the feces of rats and mice for a week or two after a feeding of spore-containing muscle. Some authors believe that certain stages of development take place in the intestinal wall after an experimental feeding and then the parasites migrate to the striated muscles where they make their appearance as "Miescher's tubes" 30 to 50 days later. It is also believed that sarcocysts may break down releasing the spores to infect other muscle cells or to be transported to other parts of the body by the blood stream to produce infective stages which may find their way out of the body.

Sarcocysts are reported to contain a toxin called sarcocystin[43] or the Sarcosporidiotoxin.[65] It is said to be a neurotoxin, thermolabile, filterable, soluble in physiologic salt solution, and toxic for rabbits but not for other animals.[65]

The pathologic effects of sarcosporidiosis are matters of dispute but it is generally admitted that *S. muris* is pathogenic or even lethal in cases of heavy infections in rats and mice.

Most surveys of parasites of wild rats fail to mention *Sarcocystis muris*. However, a 7% incidence was reported in 100 wild Norway rats caught in Washington, D. C.,[52] 10% in 100 wild rats caught in St. Louis[66] and 10.8% in 1220 rats caught in Baltimore.[2]

Sarcocysts occur in the muscles of a number of animals, especially sheep, cattle, and other ruminants; also in horses and in a few birds, especially ducks. Feeding experiments have produced conflicting results but there is much evidence that the same species of Sarcocystis may infect a number of different hosts. (For reviews of literature see references 64, 71, and 60.)

Careful scrutiny of skeletal muscles of rats with a hand lens will often reveal the slender white sarcocysts, if present. Muscles fixed in the usual way (e.g., with Zenker's fluid), sectioned and stained with haemalum or other histologic stain, will reveal the sarcocysts. The spores strain more chromatically than the surrounding tissue.

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21

Metazoan Parasites of the Rat

By

HERBERT L. RATCLIFFE

INTRODUCTION

UNDER natural conditions the two common species of rats, *Mus norvegicus* and *Mus rattus* may be infected by parasitic organisms of the phyla Platyhelminthes, Nemathelminthes, and Arthropoda. Some of these parasites are distributed throughout the world, while others are much more localized. Complexity of life cycles has been an important factor in limiting their spread.

With certain parasites of rats, host-specificity is pronounced. Others lack this to a great degree, these animals being but one of several species that may be invaded. Among the latter are organisms that parasitize man. Further, fleas and mites of rats are vectors of important diseases of rodents and of man, and, at times, control of these may constitute a definite public-health problem. Whatever may be their relations to their hosts and their actual or potential importance to the welfare of the human race, these parasites of rats are valuable material for investigation. In morphology and biological characters they are comparable in many respects to related forms that occur in other mammals, and, in general, they are easily transferred to and maintained in laboratory rats, *M. norvegicus albinus* and related strains, under controlled conditions. Thus for experiment or demonstration of certain fundamentals of parasitic adaptations these species are unexcelled.

Because of these characteristics, metazoan parasites of rats have been the subject of many studies. Material assembled in this section is, in general, taken from the more recent publications in this field. The organisms to be discussed are believed to be a fairly complete list of recognized species which may be found in wild rats with any degree of frequency. Space does not permit extended descriptions of the various organisms to be discussed. Nor are these necessary since they are easily available elsewhere.

TREMATODA

Trematode parasites of rats are relatively uncommon, only a few species having been described. These are grouped in the families Echinostomatidae and Heterophyidae, the adults of which live within the small intestine of their hosts. Another characteristic of these flukes is a considerable lack of host-specificity.[23, 24, 64, 65, 66, 69, 71, 72]

Echinostomatidae of rats are *Heterechinostomum magnovatum* and three species of *Echinostoma* (Euparyphium), *E. murinum, E. ilocanum* and *E. guerreroi*. These are small worms, the first measuring approximately 1 x 0.2 mm. The remaining three vary between the following dimensions: 2-8 x 0.3-1.5 mm. *H. magnovatum* has been found in wild rats collected near New York City. Species of *Echinostoma* have been reported chiefly in rats (and other mammals) of the Philippine Islands. Incidence of infection is said to be less than one per cent. Life history studies have shown that certain snails harbor all larval stages of *E. murinum* and *E. ilocanum*. *E. ilocanum* is a well-known parasite of man.

Heterophyidae of rats are *Ascotyle* (*Parascotyle*) *diminuta*, *Cryptocotyle lingua, Monorchotrema taihokui,* and *M. taichui*. The first of these has been reported in rats captured near New York City and was associated with *H. magnovatum*. It measures approximately 0.3 x 0.1 mm. The second is a parasite of fish-eating birds and mammals of Europe and North America. Wild rats, gulls and terns may carry the infection. Adult forms measure about 1.3 x 0.4 mm. The species of Monorchotrema have been recovered from fish-eating birds and mammals in the Orient and albino rats are easily infected. These small flattened elongate worms are less than one millimeter in length.

Experimental infection of rats by the blood flukes, *Schistosoma japonicum* and *Schistosomatium pathlocopticum,* have been reported also.

CESTODA

Cysticercus fasciolaris,[11, 12, 13, 47, 48, 49, 50] the larval stage of *Taenia crassicollis* of cats, is a common, widely distributed parasite of wild and laboratory rats.

The fully-developed form is found in the liver as an ivory-white cyst, approximately one centimeter in diameter containing a segmented worm suspended in fluid. When eaten by cats this matures in the small intestine passing ova into the feces of its host. These in turn are infective for rats, the embryos being liberated from the shells in the small intestine. Once free, they make their way into the gut wall and pass to the liver by the blood stream.

These larvae are of particular interest because malignant and benign connective tissue tumors may develop from the capsules some 12-15 months after infection. Certain strains of rats are much more susceptible to the development of these tumors than are others.

Of further interest also is the fact that immunity to this organism may be induced by several methods, among which are injections of extracts of either the larvae or the adult worm. Immunity, thus developed, persists for a considerable fraction of the life of the animal and may be passively transferred.

Cestodes—Adult Forms. The common adult tapeworms of rats are *Hymenolepis nana* (*H. nana var. fraterna* or *H. fraterna*) and *H. diminuta*, both of which are distributed throughout the world. *H. nana* of rats and other

Muridae probably is identical with that of man, but the murine parasite sometimes is designated as a distinct variety or species, being listed as *H. nana var. fraterna* or as *H. fraterna.* Here no distinction will be made.

Hymenolepis nana[3, 31, 36, 62, 75] is peculiar among common cestodes of mammals in having become adapted to life within a single host. The mature worms, narrow, elongate, and bandlike, live in the lumen of the distal portion of the small intestine. They measure 2 to 5.5 cm. in length. The scolex is globular, rostellum armed, and strobila composed of some 200 proglottides which are much broader than long. Ova measure 40 to 60 microns in length and are passed free in the feces. They are directly infectious and lose viability after some ten days even under optimum conditions. Once returned to the intestine with food or water they quickly hatch and oncospheres enter the villi of the proximal and middle segments of the small intestine within 10 hours. Cysticerci are developed within 4 days and leave the tissue to mature in the lower small intestine within 11-16 days. Loss of adult worms from the intestine begins within 13 days and usually none remain after 7 weeks unless reinfection is permitted. Since ova are passed in large numbers and only need to be returned to the mouth to continue the infection, rats do not free themselves easily of the parasite unless fecal contamination of food and cages is prevented.

Less than 1% of ova develop in rats under experimental conditions and, if large numbers are given, the host may react to prevent complete development in the gut. Young rats show passive resistance during the nursing period and for a short time after weaning, and if infected, develop active immunity after some 5 months of age. Resistance to infection is affected adversely by inadequate diets.

Strains of *H. nana* derived from various species of mammals exhibit some degree of host specificity, a factor that should not be overlooked when dealing with this and other parasites that occur in several hosts.

Although *H. nana* does not require an intermediate host, the cysticercoid stage may develop in fleas, *Pulex irritans, Xenopsylla cheopis,* and *Ctenocephalus canis* and in species of meal-worms, Tenebrio.

Hymenolepis diminuta[7, 18, 33] measures 2 to 6 cm. in length. The scolex is small, and unarmed; the proglottides are similar to those of *H. nana.* Ova are found free in the feces and measure 54 to 86 microns in length. The ova, when ingested by any of a number of grain-eating insects, fleas and certain other invertebrates, develop into cysticercoids. When these infected insects are eaten by rats, development is completed in the small intestine in about 18 days.

From wild rats examined in the Orient species of another form of cestode have been described. At first these were grouped into the genus Raillietina, but more recently have been placed in the genus Kotlania.[39, 70]

NEMATODA

Nematode parasites of rats include a considerable number of species many of which have been carefully described and illustrated in the monograph by

Hall[32] which will serve as a guide to the morphology of these forms. Complete descriptions of other organisms will be found in papers to which reference is made in dealing with them.

Strongyloides ratti,[28, 29, 30, 53, 57, 58, 59, 60] a small hair-like worm averaging some 2.5 mm. in length, is cosmopolitan in distribution. This species is distinctive among nematodes of rats because only females are parasitic. These inhabit the upper segment of the small intestine, usually entering the mucosa. Ova are produced which, as a rule, hatch in the host's intestine, larvae being found free in the feces. These may develop either into free-living males and females from which infective larvae are produced, or directly into infective larvae. Some strains of the worms show pronounced adaptation to the direct cycle; others to the indirect cycle. Infective larvae enter the body by penetrating the skin, or, experimentally, they may be introduced into the subcutis, body cavities, or stomach. Once in the body the worms pass by the blood stream to the lungs, where they undergo a phase of development. Then by way of the trachea, larynx, and esophagus they enter the intestine to complete maturation.

Several investigations have shown that rats may be rendered immune to this parasite by single or repeated infection or by injections of antigenic material derived from the worms.

Trichinella spiralis[32, 43, 44, 45, 46] also is world-wide in distribution and is transmitted from rat to rat solely by cannibalism. It is believed that rats play some part in maintaining the parasite in swine, the swine killing and eating rats about farms or stock-yards and rats feeding on fresh pork either in domestic or commercial slaughter houses.

The male of this species is small and slender, measuring about 1.5 mm. in length. The adult female is some 3 times this length, but also slender. In general, the more numerous the worms in the gut the smaller their individual size. Larvae that have encysted in striated muscle for more than 30 days are infective. How long they remain viable in the living animal is unknown. When taken into the stomach they are liberated from their cysts and develop to maturity in the small intestine within 72 hours. Fertilized females enter the intestinal mucosa and larvae are passed from the genital tract into the tissue spaces, from which they enter the blood stream to be carried to the voluntary muscles.

Development of worms within the gut is accompanied by a host response, the severity of which depends upon the numbers present. Seventy or more larvae per gram of body weight of the rat will cause death from acute intestinal disease. Entrance of larvae into the muscles produces an acute inflammatory reaction and may lead to death if parasites are sufficiently numerous. Small numbers of larvae given by mouth and developing to maturity in the gut, cause the development of a high degree of immunity within a period of about a week. In immune rats, worms fail to complete development, being lost rapidly from the intestine. Injection of trichinella antigen also will produce immunity. Vitamin-A deficiency is reported to cause a break-down of resistance.

Trichosomoides crassicauda,[32, 67, 77] a parasite of the urinary bladder, is widely distributed among wild rats. The female of this species is elongate and thread-like, measuring 10 x 0.2 mm. in length. The male is much smaller, 1.5 to 3.5 mm. in length with the body very thin and delicate. The adult male usually is found within the genital tract of the female, but may wander in and out. Ova are thick-shelled, golden-brown with polar plugs and measure 60-70 x 30-35 microns. These are passed in the urine, and when returned to the stomach in food or water, hatch there. Within a few hours after hatching larvae may be found in the lungs or body cavities. After a period of 3-6 weeks they become established in the bladder, invading the mucosa and causing proliferation of the lining epithelium. The path of migration does not seem to have been established.

Trichuris muris[32] apparently is relatively uncommon (see section on parasite surveys of wild rats). The adult worms live in the cecum, the thin hair-like anterior portion of the body being inserted into the mucosa. The male measures about 16 x 0.3 mm.; the female about 25 x 0.4 mm. Ova are passed in the feces and are brownish oval bodies with polar plugs and measure 57-62 x 30-35 microns. The life cycle is direct, ova becoming embryonated after a short period in moist soil.

Capillaria (Hepaticola) hepatica[8, 26, 32, 41, 42] probably is much better known by encapsulated masses of ova under the capsule of the rat's liver than by actually finding the worms in the hepatic tissues. Ova may be recovered from the liver by tearing the tissues apart, by digesting the tissue artificially or by feeding the tissue to rats or cats and concentrating the feces of these animals. Ova are brownish in color, oval with polar plugs and measure 56-70 x 29-43 microns. Once recovered from the rat's liver they become embryonated in 4-6 weeks if incubated in shallow dishes of water at 26 to 30°C. Embryonated ova are infectious, larvae hatching in the intestines, and passing through the wall of the cecum to the blood stream and body cavity and migrating to the liver in 2 to 10 days. Development is completed in about 30 days and ova are deposited in the liver. Adult males measure about 3.7 x 0.1 mm.; females, 78 x 0.2 mm.

This species is said not to mature in older rats, the worms that reach the liver being isolated by an inflammatory reaction.

Capillaria annulosa[32] is a relatively uncommon parasite. Adults are found in the small intestine. Males measure 14 x 0.04 mm.; females, 21 x 0.6 mm., and ova 51 to 57 microns in length. The ova resemble those of *C. hepatica.*

Heterakis spamosa,[32, 74] a fairly common and widely distributed nematode of wild rats, inhabits the cecum and proximal colon. Males measure 6.4-9.8 x 0.2-0.26 mm.; females, 7-13 x 0.68-0.74 mm., and ova 55-60 x 40-55 microns. Shells of the ova are thick and mammillated.

When ova are incubated at 30°C in water to which a trace of formalin is added, embryos complete development in 14 days. Introduced into the rat's stomach they hatch, and localize in the cecum where they grow to maturity in 26-47 days. Infections persist for 150-300 days and render the host resistant to this species.

Syphacia obvelata,[32, 56] an oxyurid nematode, has been found in the cecum and colon of a number of rodents. It does not seem to be a common parasite of rats. The male measures 1.3-1.6 x 0.11-0.13 mm.; the females, 4-6 x 0.11-0.27 mm. The ova are thin-shelled reniform bodies measuring 110-142 x 30-40 microns. This and the following species do not appear to have been completely studied.

Aspicularis tetraptera[32] is another oxyurid nematode of wild rats and other rodents found in the cecum and large intestine. Dimensions of the male are 2-2.5 x 0.15-0.17 mm., of the female, 2.5-4 x 0.19-0.25 mm. and of the ova 84-90 x 34-40 microns. Ova are typically oxyurid in shape. This species does not seem to be a common parasite of wild rats, according to recent reports from various parts of the world, but it has been found in several localities in Europe and North America.

Heligmosomum braziliense,[21, 32] a small hair-like nematode, localizes in the small intestine of its host. The male measures 2.6-2.8 x 0.09-0.1 mm. and its posterior end terminates in a large trilobed bursa. Dimensions of the female are 3.5-4.0 x 0.13-0.16 mm. Ova are ellipsoidal, measuring 63 x 28 microns. This species has been reported from Brazil and England, but surveys from other parts of the world do not include it.

Nippostrongylus muris[2, 15, 16, 17, 27, 38, 51, 54, 55, 76, 78] is a small, coiled or curved red-brown, hair-like nematode found either in the lumen or in the mucosa of the duodenum. Males measure 3.4 x 0.085-0.1 mm., females 4-6 x 0.9-0.12 mm., and ova 58 x 33 microns. The ova are ellipsoidal with very thin shells. N. muris is distributed throughout the world in wild rats, incidence being fairly high in certain localities.

Under experimental conditions, ova become embryonated within 24 hours and the larvae complete development within 7 days. Ova may be cultured by spreading feces on moist filter paper or by mixing feces with sterile moist bone-charcoal and incubating in the dark at 24°C. Larvae may be isolated from these cultures by the Baermann apparatus.

Infective (filariform) larvae enter the body of the host by way of the skin but are infectious by mouth also. After entering the body, they are carried by the blood to the lungs within 20 hours where they remain for 35-65 hours and undergo further development. Migration from the lungs to the intestine is by way of the trachea and esophagus. The gut is entered within 45-65 hours after infection. Sexual maturity and ovulation occur from 4-6 days after entering the body.

A number of studies have shown that rats may be made immune to *N. muris* by infection, that immunity is passively transferable, and that immunity may be manifested locally in the gut or generally by action of serum and leukocytes on the invading larvae.

Rictularia tani[32, 35] is one of a number of species of nematodes belonging to this genus that have been reported from rodents, but *R. tani* is the only one from rats for which a description has been found. This species is known only by the female which measures 18.5-28 x 0.6-0.7 mm. This organism probably has an insect intermediate host.

Gongylonema neoplasticum[6, 9, 25, 79] is a parasite of the forepart of the stomach of rats. It usually burrows into the mucosa. This species is cosmopolitan and incidence is often high. Males measure 15-20 x 0.11-0.13 mm., females 60-80 x 0.17-0.32 mm. and ova 60 x 40 microns. Ova, when eaten by species of Periplaneta, Tenebrio, and coprophagous beetles, hatch and mature the larvae enter and develop in the body cavity of these insects. When, in turn, these intermediate hosts are eaten by rats, maturation occurs in about 40 days. This nematode attracted considerable attention at one time when it was found to cause neoplastic proliferation in the stomach of rats, this being the first instance of experimental cancer production. Several unsuccessful attempts were later made to link it to gastric carcinoma of man.

Protospirura muris, P. bonni, P. Columbiana[10, 19, 32] and several other species of this genus are parasites of the stomach of rats, but are not frequently reported. All are similar in that they require intermediate hosts which are the same as for *G. neoplasticum*. The following measurements are given: *P. muris*: male, 14-28 x 0.5-1.0 mm., female, 15-40 x 1.0-1.75 mm., ova, 56 x 30 microns; *P. bonni*: male, 31-34 x 0.69 mm., female, 48-57 x 10.0-1.3 mm., ova, 55-60 x 40 microns; *P. columbiana*: males, 22-43 x 0.6-1.2 mm., females, 45-100 x 0.8-2.4 mm., ova, 52-58 x 30-32 microns. Ova are embryonated when passed and have thick shells.

Acanthocephala

Moniliformis moniliformis[63, 68, 73] is a fairly common parasite of rats and may infect man and other mammals. This parasite bears only a superficial resemblance to true nematodes and for description of the group to which it belongs the paper by Van Cleave[63] should be consulted. *M. moniliformis* has a thick rounded or flattened annulated body. Males measure 60-80 mm. in length, females, 100-270 mm., ova, 124-127 x 71-74 microns. The anterior end of the worm terminates in a proboscis upon which are rows of hooks. Ova are embryonated when passed in feces and develop in the same types of insects which serve as intermediate hosts for *G. neoplasticum*. The worms localize in the small intestine of infected vertebrates.

Arthropoda[4, 18, 20, 22, 33, 34]

Rats may be parasitized by mites (Acarina), lice (Anoplura), fleas (Syphonaptera) and bed-bugs (Hemiptera). For the differential characteristics of these organisms monographs and texts may be consulted. Here attention will be directed chiefly to measures for the control of these organisms.

The common species of mites that may be found on wild rats and that may infest laboratory animals are *Echinolaelaps echidninus*, the vector of *Hepatozoon muris*, *Liponyssus bacoti*, a vector of typhus, and *Notoedres notoedres*, an "itch-mite" which invades the skin.

E. echidninus is distributed throughout the world, while *L. bacoti* is found mainly in warmer climates. These organisms require several blood meals to complete their life cycles, after each feeding leaving the host and hiding in or about the "nests" or cages. Ova are deposited in straw or other litter and hatch into nymphs which suck blood as do the adults.

N. notoedres does not need to leave the host to complete its life cycle. Females burrow beneath the skin, causing inflammation and itching. Ova are deposited in the burrow where they hatch, complete development and copulate. Infected animals spread the parasite by contact.

The common louse of rats is *Polyplax spinulosa*, the vector of *Bartonella muris*. Ova or nits are attached to the hair. Nymphs which hatch from these closely resemble the adult. They also suck blood and mature in about 2 weeks. Thus the life cycle may be repeated indefinitely on one animal. Spread is by contact.

Fleas of several genera, Pulex, Xenopsylla, Ctenocephalides and Ceratophyllus, are common parasites of wild rats, in various parts of the world. Ova are deposited in the floor of burrows and hatch into worm-like larvae which feed on organic matter. Each moults three times before spinning a cocoon in which pupation occurs and from which the adult emerges.

Bed-bugs that occur in colonies of laboratory rats are usually *Cimex lectularius*, the common parasite of man. Ova are deposited about cages or in crevices of the wall or floors where adults hide after taking a blood meal. Larvae hatch in about 2 weeks and resemble the adults. They pass through 5 stages before maturing, and during each stage require a meal of blood. The female also must feed on blood before eggs may be developed.

Control of these arthropods in colonies of laboratory rats varies somewhat with the species. The two species of mites, *E. echidninus* and *L. bacoti*, and the louse, *P. spinulosa* must be eradicated from cages, bedding, colony rooms and buildings, and from the hosts. Cages and bedding for the animals may be subjected to steam or to dry heat, 60-75°C for 30 minutes, which kill all stages of the organisms. Rooms must be sealed to prevent escape or entrance of the parasite and the building made proof against wild rats. For buildings or for single rooms cyanide gas, applied efficiently, would be the most effective measure, but insecticidal sprays are also effective.

Rats infested with these parasites may be sprayed or brushed with insecticides to kill any organisms that remain on the body. Volatile oils containing derris or pyrethrum should be used. Derris powder dusted into the fur and bedding also may be effective. The entire skin surface must come in contact with these substances in order to kill all parasites.

When animals are infested with the "itch-mite," *N. notoedres*, acaricidal ointments must be applied directly to the lesions at intervals of 5 days. A suspension of sulphur in olive oil or other light vegetable oil in the same proportions as in sulphur ointment is easily applied and has proven very effective with a related type of mite, Otodectes. In addition to treating individual animals, cages, bedding and rooms must be sterilized. Except with very valuable animals, replacement probably would be more economical than attempting treatment.

Fleas rarely are a problem in experimental colonies of rats unless the building is infested with wild rats and mice, and the bedding in the cages allowed to remain unchanged for weeks. If colony rooms become infested by fleas, thorough cleaning, followed by insecticidal sprays, will be effective.

Bed-bugs, however, are sometimes a difficult problem, but, if the colony rooms are cleaned and sprayed well with insecticides, and cages and bedding sterilized by steam or dry heat, these can be eliminated.

AVAILABILITY OF PARASITES OF RATS[5, 14, 40, 52, 70]

Listed in Table XXIII are percentage incidences of common Cestodes and Nematodes of wild rats, usually *Mus norvegicus*, which have been examined within recent years in various parts of the world. This summary will indicate in a general way availability of organisms to one who may wish to obtain material for demonstration or experiment. With one exception these surveys did not include Arthropods, probably because the rats had been killed some hours before autopsy. Few ectoparasites remain long on the body of the host during life and usually leave it soon after death.

TABLE XXIV

Percentage incidences of common cestodes and nematodes of wild rats

Parasite	Locality				
	District of Columbia	Philippine Islands	Toulon, France	Baltimore, Maryland	Tippecanoe County, Indiana
Cysticercus fasciolaris	54		6	19 2	
Hymenolepis nana	41	1 7	23	14.4	7.5
Hymenolepis diminuta	31	64.	24	16.6	7.5
Strongyloides ratti		74.		20.2	
Trichinella spiralis	3			1.2	2.5
Trichosomoides crassicauda	73.	74.		90.4	
Trichuris muris			5		2 5
Capillaria hepatica	77	90.	1.2	85.6	
Heterakis spumosa	49			1.6	
Syphacia obvelata				0 48	
Nippostrongylus muris		58.		16 8	75 0
Gongylonema neoplasticum	5	44.			
Protospiruia (Sp)		1.3	20.		
Moniliformis moniliformis	4	4.2		0 64	

COLLECTING MATERIAL[1, 4, 20, 37, 43, 51, 67]

If wild rats, collected for examination, be captured alive they should be kept separately in small wire cages to prevent fighting and to allow individual fecal examination. Feces may be examined by direct smear or may be concentrated according to any of several methods that have been described elsewhere. Urine may be collected by keeping the animals in small cages, the bottoms of which are screened to prevent food and feces contaminating the sample, and are placed over glass funnels emptying into suitable containers. Ova of *Trichosomoides crassicauda* will be found in the sediment of the urine. Before autopsy, food should be withheld for 24 hours to empty the gut as much as possible and facilitate examination. Autopsy should be done a few hours after death when the gut has relaxed and any organisms that burrow into the mucosa will have returned to the lumen.

For postmortem examination the stomach and intestines should be removed intact, then separated into the natural segments each of which is studied separately. Also the liver and urinary bladder should be removed intact.

Segments of the digestive tract may be examined, after opening each and covering the mucosa with normal saline, with a hand lens or dissecting microscope. The contents of the gut should be broken up to obtain any worms that have become enclosed in this material.

Another method that may be recommended is flushing segments of the gut with warm water, collecting material from each in a suitable flask for concentration and examination.

The liver may contain *Cysticercus fasciolaris* and ova of *Capillaria hepatica*, the characteristics of which have been described.

Examination of the urinary bladder should be done according to the first method suggested for the gut.

The diaphragm may be cut into pieces of a suitable size, pressed between slides and examined with the dissecting microscope for cysts of *Trichinella spiralis.*

Fleas, lice, and mites may be collected from the bodies of newly-caught wild rats by placing the animals in a chloroform jar until dead. Chloroform kills these parasites as well as their hosts. Freshly killed rats may be placed in a paper bag and the parasites taken as they leave the body.

Larvae of fleas may be collected from screenings of rat burrows, and, if the bedding from these is placed in paper bags, which then are sealed for 2 or 3 days, mites and lice will feed readily on any small animal introduced into the bag. The organisms may be collected after they have engorged.

For identification of ova, larvae, and adults of the various parasites that occur in or on rats, and for special methods of preserving, culturing, or propagating the infections, references to the specific forms should be consulted.

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22

Spontaneous Diseases of Laboratory Rats

By

HERBERT L. RATCLIFFE

In the usual course of events a number of diseases may be expected to develop in colonies of laboratory rats. Some of these will be due to microorganisms; others will be of types for which no definite etiology is known. The incidence and variety of these morbid conditions may vary with different colonies and with different strains within the colonies. Adequate explanations have not yet been advanced for the apparent differences in susceptibilities.

This chapter has been prepared with a view to facilitating recognition of the more common spontaneous pathologic conditions that may develop in the usual breeding stock of rats, so that these may not be confused with the effects of experimental procedures. Whenever possible, discussions of bacterial diseases include an outline of control measures which may be applied. Rare and unusual abnormalities, as well as infections of wild rats, such as plague and leprosy, have been omitted. Omitted also are parasitic and nutritional diseases which have been considered in other chapters.

Spontaneous diseases known to occur with any degree of regularity in laboratory rats may be considered under the following subdivisions: 1. Specific and nonspecific infections; 2. Neoplasms; and 3. Diseases of the heart, blood-vessels and kidneys. While this outline may not correspond to the usual discussions of diseases of lower animals, it seems best suited to the present limited knowledge of the subject.

The infectious diseases and neoplasms of rats are much better known than are other morbid processes such as arteriosclerosis and its sequelae. The reason for this is clear. Pathogenic bacteria and certain animal parasites may contribute considerably to morbidity and mortality of a colony, interfering with breeding and other experimental studies. Some of these disease processes of rats are similar to infections of other animals or the invading organisms may be the same even though the disease may differ with the species of host. In such instances the rat has been a convenient test subject. Similarly the various forms of neoplasms of rats have been investigated for their possible contribution to the general problem of tumor development.

SPECIFIC INFECTIONS

Paratyphoid Disease. This is the only specific infection at all common in laboratory rats, excepting, of course, certain conditions due to animal parasites. How common paratyphoid disease may be in various colonies in this country is not known, but there is reason to suspect that it is much more common than is generally realized, and that, in spite of adequate descriptions of the characteristic morbid changes, it goes unrecognized. In fact the lesions of paratyphoid have been attributed to experimental procedures on more than one occasion in recent years.

Paratyphoid disease of rats is caused by bacteria of the genus Salmonella, several species of which have been incriminated. Löffler, who in 1892 first recognized the nature of this condition and studied it in mice, named the invading organism Bacterium typhi murium. Later investigations have shown that the two most common species of bacteria involved are S. enteritidis and S. aertrycke,[1] which may appear together or singly in animals that develop the disease spontaneously. These bacteria sometimes cause intestinal disease in man, being ingested with contaminated food. Wild rats are often carriers of the organisms,[2] and one source of infection for them is the so-called "virus" preparations advocated and used for their control.[2] Thus colonies of laboratory rats may become infected by food or by contact with the excrement of wild rodents.

Paratyphoid infection may run an acute course with high mortality, or may be relatively mild and chronic. Both forms of the disease may occur in the same colony, but the chronic, subclinical form probably is the more common.

In rats developing the acute form of paratyphoid weight is lost rapidly, ears become pale, eyes take on a chocolate-brown tinge, brownish crusts form about the nose and occasionally diarrhea is seen. Examination of the blood reveals severe anemia.[3] At postmortem examination, the liver is pale and friable, and sometimes mottled. Beneath the capsule and on the cut surface minute yellow-brown foci of necrosis, hardly visible to the unaided eye, are seen. The spleen is enlarged, its capsule tense, the pulp dark purple, and irregularly mottled by small hemorrhagic foci and yellow-gray areas of necrosis. The intestinal wall is not regularly involved, but may be injected, with occasional hemorrhages in the mucosa. The lymphoid tissue of the gut may be hyperplastic and sometimes ulcerated. The mesenteric lymph nodes usually are unchanged, but the cervical and mediastinal lymphoid tissues are regularly swollen and soft. Within the lungs purpuric spots are the only lesions found, although pneumonia may be coincident with paratyphoid. Within the heart muscle occasional, irregular, pale areas of necrosis, sometimes develop and seem to be part of the response to the disease.

Microscopic examination of the liver finds increased numbers of mononuclear leucocytes throughout the capillary bed which also contains scattered fibrin thrombi. Scattered through the parenchyma are small, usually circular, foci all under one millimeter in diameter in which tissues are converted into eosin-staining material which is stippled with nuclear fragments.

In the spleen, lymphoid cells are everywhere undergoing fragmentation, mononuclear phagocytes are increased, sometimes greatly so, and necrotic areas, similar to those seen in the liver, are distributed in the pulp. Within the bone marrow extensive necroses also occur. The cervical and mediastinal lymph nodes undergo changes similar to those of the spleen but the mesenteric nodes are involved slightly, if at all.

The heart muscle may show more or less extensive interstitial accumulation of monocytes, and scattered areas in which the muscle has undergone degeneration. The alveolar walls of the lungs may be more cellular than normal but more severe changes are lacking. The kidneys and the intestine are unchanged.

When rats develop the chronic form of paratyphoid there may be no obvious evidence of illness.[4] Breeding and growth records may be satisfactory although a considerable percentage of the stock is infected. The lesions of this type of paratyphoid disease differ in some respects from those seen in the acute phase. The cecum is enlarged and distended, its wall is thickened and inelastic, and its mucosa presents irregular, elongate ulcers which may measure up to 2 cm. in length. The floors of these are covered with thin crusts. The lymphoid tissue of the ileum is hyperplastic but usually not ulcerated. The mesenteric lymph nodes are enlarged and soft, with cysts often developed in them. The spleen may be moderately enlarged, with dark red, uniform pulp which swells on section. The gross appearance of the liver is not abnormal.

Microscopic examination of the cecum reveals evidences of chronic inflammatory changes with imperfect repair about the ulcers, and extensive edema and fibrosis of the wall. The mesenteric lymph nodes contain moderately hyperplastic follicles, show occasional necrotic foci, and often are distorted by the development of lymph cysts. Scattered necrotic areas occur in the spleen and liver also, but otherwise these organs present few changes.

More recent studies of chronic ulcerative cecitis of rats by Stewart and Jones[31] indicate, however, that this disease may not be a part of paratyphoid infection. At least it did not follow experimental infections, although they report isolation of a strain of Salmonella from some of the animals in which there were ulcers in the cecum.

From personal experience it is believed that rats which develop the subclinical type of paratyphoid disease may experience more extensive morbid changes within the liver and spleen when they are subjected to debilitating influences. Admittedly this has not been subjected to a strict experimental test, but has been seen with sufficient regularity to be offered as a suggestion to those who may be working with infected animals.

During the course of acute and fatal paratyphoid infections the bacteria may or may not be passed in the feces, and the organisms may persist in the tissues of the host without producing disease.[5] Probably the chief sources of continued infection in a colony are animals that do not develop symptoms. In one colony in which growth and reproduction records were equal to set standards, 60% of the animals were excreting the bacteria in the feces.[4]

Control of paratyphoid of rats probably can be accomplished by vaccination with heat-killed suspensions of several types of Salmonella as has been done with guinea pigs.[6]

The technic for isolating Salmonella from feces or tissues and for theii identification is well standardized and may be found in any comprehensive treatise on bacteria.

Pseudotuberculosis. Another disease which, in its gross appearance, is sometimes confused with paratyphoid infection is pseudotuberculosis of rodents, particularly of guinea pigs.[7, 8, 9] Wild rats and mice sometimes are infected also. The etiological agent, Pasteurella pseudotuberculosis, is similar in many respects to the plague bacillus. This organism usually is not pathogenic for white rats.

NONSPECIFIC INFECTIONS

Middle Ear Disease and Labyrinthitis. In a recent discussion of bacterial infection of the middle and inner ear of rats, King[10] suggested "labyrinthitis" as an appropriate designation since the characteristic disequilibrium indicates involvement of tissues of the labyrinth. The number of animals that exhibit symptoms of labyrinthitis usually is a small fraction of those in which infection of the tympanic cavity occurs, and disease of the inner ear seems to be an extension of the inflammatory process from the middle ear into adjacent structures. Hence the dual heading for this section.

Recognition of suppurative disease of the middle ear is possible in the living animal only when labyrinthitis has developed also, in which case characteristic behavior changes are seen. The following description of these is quoted from Dr. King's paper: "The clinical signs of labyrinthitis are definite and distinctive. The first indication of its presence is a slight tilting of the head to one side. Subsequently the rat holds its head continually close to the floor of the cage with the nose pointing away from the medial line. Lack of coordination in muscular movements is shown by the fact that the individual is unable to run in a straight line, but does so in a curve. It may stagger as it runs and if it falls on one side has difficulty in regaining the upright position. When suspended by the tail the body invariably rotates rapidly. Because of this latter characteristic rats with labyrinthitis are often called 'twisters.'"

At postmortem examination, the extent of the disease may vary with the animal. When clinical signs have been evident, inflammatory changes will be found in the structures of the inner ear, as well as in the tympanic cavity. From the inner ear the disease may extend into the petrous bone and auditory nerve. In one or both ears grayish-yellow pus of varying consistency within the auditory apparatus is the most obvious indication of disease.

The incidence of middle ear disease and of labyrinthitis varies with the colony.[11] In some, about half of all adult animals have shown infection of the tympanic cavity when autopsied. Degree of susceptibility also may vary with the colony. Usually rats under 3 months of age are not infected, but from this time on the infection rate increases rapidly until the eighth month after which it is fairly uniform. As a rule the incidence of labyrinthitis is much lower, usually less than 4% of all animals in which the middle ear is diseased. Dr. King's inbred strain of albino rats were, at one time, an exception, having a much higher rate of disease.[10]

Bacteriological studies of middle ear disease are inconclusive, a variety of micro-organisms having been isolated. Sometimes these may be present in pure culture or may be mixed in various combinations. Often also the exudate in diseased ears is sterile: 44% of young animals and 12.5% of adults in which purulent exudate was found in the middle ear were of this type in one study. It has not been possible to correlate inflammatory changes and bacterial flora of the nasopharynx with the occurrence of middle ear disease.[12, 13]

Two methods have been developed and are recommended for control of this disease of rats.[10, 14] In one instance young breeding rats under 6 months of age were paired in small cages, in quarters separated from the original colony, and allowed to mate. The males were autopsied at once and the females, after the young were weaned. Only young from parents found to be free of middle-ear infection were saved. By following this procedure through 7 generations a strain free of the disease was established. The second method was to separate young from their mothers immediately after birth, before the placenta was removed, and allow them to be reared by foster mothers from a strain in which middle ear disease and labyrinthitis was very rare or unknown.

Pneumonia. Inflammatory and suppurative diseases of the lungs probably are the most common of all morbid conditions of adult rats. The incidence of pulmonic lesions increases with age up to about one year at which time 75% of the animals may be involved. Animals that are under one year of age less frequently show evidences of pneumonia and the disease is rare before the third or fourth month.[11]

Definite signs of pneumonia usually are not evident until the disease is fairly well advanced. Indeed, one is often surprised at the extent of lung involvement to be found in well nourished animals. However, as the disease becomes more extensive the animals take on an unthrifty appearance. The coat is roughened, there is loss of weight and increasing respiratory difficulty. Often the rapid breathing is sufficiently noisy to be heard at a distance of several feet. At this stage the animals sit with the back arched and are relatively inactive.

The extent of pulmonary disease to be found at autopsy will vary with the animal.[15, 11] In the early, subclinical stages small foci of inflammation are found in and about the smaller bronchi of the basal lobes. Often these may appear as shot-like masses in the lung. Sections through these areas show accumulation of mucoid or mucopurulent plugs within short segments of bronchi and inflammation of the wall and adjacent parenchyma. More advanced lesions extend to the pleura as gray, or yellow-gray, firm nodular masses which may be elevated above, or lie below the general lung surface. Later, whole lobes or an entire lung may be firm, shrunken and nodular, and early changes may have developed in the opposite organ. On the cut surfaces, these lesions may have a central portion of mucopurulent exudate, which may be liquid, semisolid, or compacted into a caseous mass, surrounded by red or red-gray collapsed lung tissue, or the whole structure may have a firm, irregular fibrous character with here and there small cavities containing pus.

Occasionally purulent exudate may be found in the pleural and pericardial cavities.

On microscopic examination of the lung in the early stages of the disease, bronchi are found to be dilated and filled by secretion or exudate in which there are few leucocytes, and peribronchial tissues show moderate fibrosis and accumulation of lymphocytes. Distal to the obstructed bronchi the pulmonary tissue is collapsed. With more advanced disease polymorphonuclear leukocytes become increasingly more abundant, filling the bronchial lumen and adjacent alveolar spaces and infiltrating the interstitial tissue. These changes may be extensive, and the appearance one of bronchopneumonia. Later, the changes of acute inflammation may be mixed with those of more prolonged disease such as considerable fibrous tissue growth, and proliferation of glandular epithelium, and the confused appearances of the lung tissue suggest the development of carcinoma complicated by infection. In other instances the dilated bronchi may form relatively large cavities in which exudate accumulates and becomes inspissated, or in which the exudate is more or less completely replaced by debris from the bronchial walls. For, in the development of bronchiectasis and abscesses, the normal mucosa of the bronchi may be replaced by squamous epithelium. This metaplasia may be limited to a segment of the wall or may be extensive and extend into alveolae, an alteration not related to vitamin-A deficiency. Within the bronchi this change may result in bizarre epidermoid formations that suggest carcinoma, but mitoses are rarely seen, and the growth is a local one. In certain reports of carcinoma of the lungs of rats however, it seems that this metaplasia of the bronchial mucosa has been mistaken for cancer.[16]

The fact that at least these two types of response of the lungs of rats to inflammatory stimuli may simulate neoplastic disease does not mean cancer is not to be expected, but, unless there be distant extension of lesions that appear to be tumors, it may be well to interpret them as aberrant attempts at heating.

Bacteriological studies of pneumonia of rats have been inconclusive. As with infections of the tissues of the ears, the organisms isolated from areas of consolidation and abscesses of the lungs have varied with the colony and in no instance has evidence been offered that any single type of bacteria is associated with the disease with sufficient regularity to assign it more importance than others. Often too, cultures of diseased lungs are sterile.[12, 13] Possibly pulmonary suppuration of rats is similar to infections of the ear in that some, as yet unknown, agent is transferred from parent to offspring to perpetuate the condition.

One suggestion has been that pneumonia of rats is initiated by air-borne bacteria which chance to enter a previously prepared focus, and that such foci are developed first in the basal lobes of the lungs when bronchi or bronchioles become plugged by mucus secretion and the air spaces supplied by these channels collapse. Once bacteria are established in such an area, acute inflammatory changes take place, further damaging the tissue and gradually spreading to involve more and more of the organ. As the chief factor in the development of mucus plugs and atelectasis which in turn lead to pneumonia, inactivity of the animals and inadequate ventilation of the lungs have been suggested.[15]

Thus perhaps control of pneumonia in rats should be directed to prevention of atelectasis. Another method suggested for elimination of this disease is to follow the procedure which was successful with ear infections, of breeding from pneumonia-free animals until a clean, and possibly resistant, strain be obtained.[14]

When, however, as sometimes occurs,[15, 17, 30] this disease can be attributed to an organism such as *Bacillus bronchisepticus*, for which antiserum is available, the infection may be cured. Intraperitoneal injection of 0.5 to 1.0 cc. of antiserum per animal, twice daily for 5 or 6 days may result in rapid recovery.[17]

NEOPLASMS

In all probability laboratory rats may be expected to develop any or all of the types of tumors that occur in other mammals. But in spite of a considerable number of studies, generalizations as to the relative susceptibility of these animals to given forms of neoplastic disease are impossible at the present time. For it seems that the frequency with which any type of neoplasm may appear varies with the colony and strain.

Descriptions of the tumors of rats are scattered and to attempt enumeration of all known varieties would be profitless. Instead, attention has been given to the more common forms of spontaneous neoplasms, their frequencies in different colonies, the age groups and sex involved, and something of the character of the lesions.

In their histologic make up the tumors of rats are similar to analogous growths of man, and criteria, upon which classification of the tumors of man is based, may be applied. Hence descriptions of morphology will not be given, these being available in treatises on general and special pathology.

Analyses of neoplastic disease within colonies of rats seem to be available for only three groups and even these do not cover a sufficient number of cases to allow estimates of susceptibility except for a few types of growths. One of these studies, published in two sections,[18, 19] contains records of tumors which developed in the rat colony of the Institute of Cancer Research of Columbia University. During the period covered by this study the colony was made up of seven distinct strains of animals. More recently a similar analysis was made of tumors that occurred in rats of the Experimental Colony Strain and the Wistar Stock Colony of The Wistar Institute of Anatomy and Biology.[20]

Table XXV shows the more common sites of tumor growth of rats of colonies maintained by the two institutions mentioned. This table does not include all cases, because occasional involvement of one or another structure did not seem of particular moment. Under each organ or organ-group tumors are tabulated according to their growth characters, i.e., benign and malignant. The cases are divided as to sex, and the approximate average age in days is given for each group of animals which developed tumors at any

TABLE XXV

Chief Sites of Primary Tumor Growth, Number of Tumor-bearers, Their Sex and Average Age, and the Types of Tumors (either Epithelial or Mesenchymal) occurring in Colonies of Rats belonging to the Institutions indicated

Sites of Tumor Growth	Two Colonies of The Wistar Institute of Anatomy and Biology								Colony of the Institute of Cancer Research, Columbia University							
	Cases ♀ ♀	Av. Age Days	Tumors E*	Tumors F*	Cases ♂ ♂	Av. Age Days	Tumors E*	Tumors F*	Cases ♀ ♀	Av. Age Days	Tumors E*	Tumors F*	Cases ♂ ♂	Av. Age Days	Tumors E*	Tumors F*
Skin and Subcutis																
Benign	2	740		2	7	750	1	6	19	600	1	18	6	540	1	5
Malignant	5	650		5	8	760	1	7	50	690	19	31	24	600	6	18
Mammary Glands																
Benign	192	650	209	2	14	660	5	9	64	690	64		1	510	1	
Malignant	14	460	12	2					4	630	2	2				
Gastro-intestinal																
Benign									1	780		1	1	390		1
Malignant	2	330	2		1	900		1	6	630		6	6	580	1	4
Liver																
Benign									1	210	1		3	480	3	
Malignant									1	600	1					
Abdominal serosa and lymph nodes																
Malignant					2	730		2	34	520		34	50	400		50
Genitalia																
Benign	5	750		5					5	630	1	4	1	570		1
Malignant	12	650	9	3	2	660		2	43	630	21	22	2	870	1	1
Kidneys																
Malignant					1	850		1	2	600	1	1				
Embryonal					4	210	mixed		3	330	mixed		1	270	mixed	
Lungs and Mediastinum																
Malignant	1	390		1	1	250		1	6	630		6	5	660		5
Thymus																
Benign									62	780	type not stated		20	660	type not stated	
Malignant	2	780	1	1	1	810	1		8	750						
Skeleton																
Malignant	3	860		2	3	560		3	1	630		1	1	690		1

E*—Epithelial in origin F*—Fibrous or mesenchymal origin given site. The growths for each of these sites are again subdivided according to histologic type, whether of epithelial or mesenchymal cells.

Differences in organ and tissue susceptibility of the different strains of rats making up the colonies of these institutions are obscured in this table. Certain of these will be mentioned later.

From the two colonies of the Wistar Institute 273 tumor-bearers, 231 females and 42 males were obtained. This was 3.1% of all animals which died during the study—a period of 5 years. For all female deaths the incidence of tumors was 5.7% and for all male deaths, 1.0%. The colony of the Institute of Cancer Research supplied 426 tumor-bearers, 301 females and 125 males, an incidence of 1.53%. For females the percentage was 2.14 and for males 0.92. In comparing the number of instances in which tumors developed in any one site in animals of the two groups as shown in the table, it should be noted that the total number of cases in the second was nearly double that of the first.

The frequency of tumor development in the skin and subcutis was distinctly higher in the colony of the Institute of Cancer Research. No malignant epithelial tumors were found in females and only one in males of the Wistar rats, but this neoplasm was common in both sexes of the second group. The relative proportions were reversed in the case of the mammary glands, which produced 77% of all tumors in females of the first group, while only 21% of females of the second group developed growths at this site. At all other sites listed, except the skeleton and kidneys, the second group led the first in the development of neoplasms. This was particularly notable in tumors of the thymus, but here differences are not so great, if benign growths, which were not recorded in the first group, be omitted.

It may be supposed that these differences in the rate of tumor development at different sites reflect differences in genetic constitution of the strains or races of rats composing the groups. The two colonies of the Wistar Institute, for example, gave different gross rates of tumor development from year to year throughout the study, the Experimental Colony strain showing the higher incidence and in only this strain of rats were neoplasms of the genitourinary organs found. Likewise distinct differences in incidence and types of tumor growths were found in the strains that made up the colony of the Institute of Cancer Research. Neoplasms of the thymus, for example, were confined to the Copenhagen strain.

As to the histologic types of tumors seen in these groups of rats, it may be noted that those developing from fibrous tissue are more numerous in both, if growths of the mammary gland be omitted. In the skin and subcutis, and intestinal tract fibromas and sarcomas greatly exceed epithelial tumors. For most other animal species these proportions are reversed. Even in the genital tract malignant tumors are about equally divided between carcinomas and sarcomas.

The most common of all tumors listed, and certainly among the most frequent tumors of rats in general, are the fibroepithelial growths, fibroadenomas, of the mammary glands of females. This type of tumor may develop in any part of the mammary tissue, usually as a single growth, but in some 10% of the cases there will be multiple sites of origin. The occurrence of fibroadenomas, in the female, has been associated with abnormalities of the pituitary and ovaries. Generally this type of tumor does not make its appearance before the age of one year, and grows slowly, but may reach a size, almost if not quite, equal to that of the host animal.[21, 22, 23] Under suitable conditions it is possible to transplant portions of fibroadenomas in series, and obtain various modifications in their histologic make up.[24] The age of the recipient animal and other factors may be responsible for this change. Female rats of the Experimental Colony Strain seem to be particularly susceptible to the development of these tumors of the mammary glands, but a much higher incidence has been reported for the "Albany Strain" of rats. Male rats of the Experimental Colony Strain of The Wistar Institute also occasionally have fibroadenomas of the mammary tissue.[20, 25]

DISEASES OF THE HEART, BLOOD VESSELS AND KIDNEYS

Pathologic processes that involve the heart, blood vessels, and kidneys need not be interrelated and of course, often are not. It is well known, certainly, that some of the diseases of blood vessels, particularly arteriosclerosis, by their effects upon blood supply, may damage the heart, kidneys, and other organs. And a reduced blood supply to the kidneys, with accompanying phenomena, may be reflected in changes in the myocardium. Since the best known of the spontaneous diseases of the heart and kidneys of rats seem to be referable to alteration in the vascular system it seems appropriate to present the material in one subsection.

More or less incidental descriptions of morbid changes of the heart, blood vessels and kidneys of rats are fairly numerous. Usually these have been given in connection with other studies. But for present purposes, a discussion of these reports does not seem worth while, for they do not indicate incidence of the diseases for the strain of rats used.

To the best of my knowledge only one detailed study of spontaneous diseases of the heart, blood vessels and kidneys as developed by a relatively large group of animals is available. This, by Wilens and Sproul in 1938, gives detailed descriptions of lesions of these organs as found at autopsy of 266 females and 221 male rats of the Osborn-Mendel strain from the colony of the Department of Chemistry of Columbia University.[26, 27] These animals were fed adequate diets, were not subjected to experiment and died of natural causes. The average age of females was 746 days and of males, 702 days. This group of animals may be supposed to present a fairly complete series of pathologic changes which would recur in other generations of the parent stock. Whether or not other strains of rats may develop similar diseases remains to be determined, but from available evidence this is doubtful.

In addition to the lesions found in the heart, blood vessels, and kidneys of this series of rats, suppurative changes of the lungs, ears, and other parts of the body were often present. These were not discussed in detail, but 75% of the animals had some grade of bronchiectasis and lung abscess. In this respect at least this strain of rats resembles many others.

Diseases of the Heart. The most common form of disease of the heart found in these animals was fibrosis of the myocardium which occurred in nearly 60% of the series. The incidence for males was 65.0% and for females, 55.3%. Males also developed the more severe forms of the diseases about twice as frequently as did females.

Myocardial fibrosis was recognized only on microscopic examination. The most common sites of involvement were the wall of the left ventricle and the interventricular septum. The wall of the right ventricle, except in the basal parts, rarely was replaced and the auricular muscle was intact. Scar formation seemed to occur most often in areas of muscle adjacent to normal masses of fibrous tissue such as is found at the base of the ventricles and about the larger branches of the coronary arteries. But the thin area of muscle at the apex of the heart was often involved also.

In these and other areas the myocardium was replaced by acellular connective tissue from which leukocytes and blood pigment were absent. Most often the replacement was discontinuous and irregular, with bands of muscle interspaced with fibrous tissue. In some cases the growth of fibrous tissue was more or less diffuse, as if interstitial tissue were increased generally. Rarely were these relatively large continuous areas of scar formation, such as occur when infarcts heal. In occasional cases foci of subacute or chronic inflammation were associated with fibrosis, but this occurred too seldom to suggest that the scar formation within the myocardium was associated with infection. Furthermore the smaller and presumably early lesions did not seem to be of an inflammatory nature.

Frequently coincident with myocardial fibrosis, and perhaps the most probable cause of it, was sclerosis of the coronary arteries. This disease appeared in these animals at an earlier age than did scarring of the myocardium and in the older groups the frequency and severity of the arterial and myocardial disease tended to be parallel. However, there were cases in which this association was absent, but had examinations been more extensive it was believed that more complete correspondence would have been seen.

Sclerosis of the coronary arteries also was found to be more severe in the male than in the female, the proportion being about 2 to 1. For all animals the incidence was 57.7%.

This change in the coronary arteries seemed to begin with irregular loss of muscle from the media, and its replacement by fibrous tissue. Some of the remaining muscle cells were found to be hypertrophied and vacuolated. Other groups of these cells occasionally formed imperfect new layers in the adventitia or subintimal tissues, giving the vessels irregular contours, thickening the walls, and narrowing the lumen. Eventually the media was replaced entirely by hyalinized connective tissue, the internal elastic membrane remained intact although straitened, but the intima was normally thin. Calcification of the fibrotic media occurred infrequently, there rarely being more than small deposits beneath the elastic lamina, but occasionally small platelike deposits were found. Associated with the changes in the media was increase in extent of the adventitial fibrous tissue. Although the walls of the coronary arteries were thickened in this process and the lumen narrowed, sudden occlusion and infarction was not found. Apparently myocardial fibrosis usually resulted from gradually decreasing blood supply, atrophy of the muscle, and slow replacement by connective tissue.

Hypertrophy of the heart, particularly of the left ventricle, also was observed in some 35% of the series. Each sex was about equally affected, and rarely was the change seen in animals under 500 days of age. In some instances explanation for hypertrophy was lacking; in others it was associated with scarring of the kidneys, or again with fibrosis of the myocardium.

Much less frequently other lesions were found in the myocardium. These included metastatic abscesses which developed in the course of spread of some acute infection, usually as a terminal event. Occasionally also infiltrations of the heart muscle by leukemic cells and other neoplasms were observed.

Other relatively rare diseases of the heart observed in this series of animals include acute and chronic inflammatory changes of the pericardial surfaces. These were unrelated apparently. In the first instance the disease seemed to be the result of extension of suppuration from adjacent structures, particularly the lungs, while irregularly distributed proliferation of mesothelial cells and subepicardial fibrous tissue, and accumulation of lymphocytes, plasma cells, mononuclear leukocytes, and occasionally eosinophilic leukocytes characterized the chronic lesions. Commonly this second type of disease of the pericardium seemed to be part of a more general one which involved several of the serous membranes such as the pleura and peritoneum. To the unaided eye these surfaces were smooth but opaque.

Much less frequent also than myocardial fibrosis were certain lesions of the endocardial surfaces. These were listed as "intracardiac thrombosis, chronic auriculitis, bacterial endocarditis and chronic endocarditis."

The most common of these was intracardiac thrombosis occurring in 6.4% of the animals. In 20 of 31 cases the left auricle was the site of thrombus formation. Only when the clots were of large size, filling the chamber and obstructing blood flow could they be recognized grossly. On microscopic examination these thrombi were of typical structure and had recently formed, there being little evidence of organization. Factors which might favor thrombus formation within the auricles could not be determined.

Chronic auriculitis developed in 3.7% of the cases. It was recognized only by microscopic examination and essentially was a chronic inflammatory process with thickening of the endocardium by fibrosis and accumulation of leukocytes in this tissue and throughout the wall of the auricle.

Bacterial endocarditis occurred also in about 3% of the series, reproducing the typical picture of this disease. In the 15 cases the mitral valve was involved 11 times. In 15 other cases mild inflammatory distortion of one or more valves of the heart was observed, a change which was termed chronic endocarditis.

Diseases of the Blood Vessels. The most frequent type of disease of the arteries of mammals generally is characterized by loss of elasticity. This occurs as age advances and may be accompanied by deposits of lipoid substances and calcium in the intima or media of the vessel. Usually the aorta seems to be involved earlier and more extensively than other vessels. In a recent monograph on arteriosclerosis of mammals and birds it was noted that rodents rarely develop these changes.[28] Observations on the rat confirm this view.

The strain of rats studied by Wilens and Sproul seldom developed sclerotic changes in the aorta. With advancing age some loss of elasticity was noted and occasionally calcium deposits formed in the media at the junction of the heart and the vessel, and again at the openings of the larger branches. Other medial and intimal changes were lacking.

With the smaller arteries, that is, the coronary, pulmonary, renal, mesenteric, cerebral, and spermatic, considerable degrees of abnormality developed however. Sclerotic changes of the coronary arteries have been described in conjunction with diseases of the heart. The more frequent lesions of the other vessels were similar to these.

As in the coronary vessels, the arteries and arterioles of the lungs of rats underwent progressive loss of muscle, with subsequent or coincident irregular replacement by ingrowth of fibrous tissue to distort the media. The change seemed to be a rather diffuse one extending throughout the arterial system of these organs. In this series of animals, the incidence of this lesion was 47%. Possibly it would have been higher with more extended examination. In another series of rats this condition developed in 12 of 75 animals.[29]

Associated with sclerosis of the pulmonary arteries deposits of calcium often formed in the inner layers of the media or in the intima and media. These calcified masses often were covered by normal or thickened intimal tissues but sometimes extended as jagged spurs not covered by endothelium, into and across the lumen. Thrombi were not formed on these. Calcium deposits also formed in the walls of otherwise normal vessels, particularly at point of branching.

Differences in calcium intake did not seem to account for the presence or absence of calcification of walls of the pulmonary arteries, nor was inflammatory disease correlated with this change.

Sclerosis and calcification of the other arteries seemed to develop in much the same fashion as in the pulmonary arteries. Wilens and Sproul[27] found the most extensive changes in the spermatic arteries where calcium salts sometimes formed rings in the media and intima. In their series of animals arteriosclerosis was rarely found in more than three organs in any one animal.

Except for the lungs the arterioles rarely presented any change. Here the walls of these vessels were thickened by hypertrophy of the muscular tissue, in association with sclerosis and calcification of arteries. This alteration also has been noted in other strains of rats.

A less common pathologic change of arteries of the rat, described for the first time by Wilens and Sproul,[27] was a form of inflammatory disease of the arterial walls comparable to periarteritis nodosa of man. This was found in 9.7% of the series, 30 females and 17 males. The average age of females was 856 days and of males 700 days. The frequency of the disease increased with age and developed in more than 15% of all over 900 days.

This form of periarteritis of rats is described as a progressive, acute or subacute inflammation. Healed and continuing lesions may be found in the same animal. Arteries of almost all organs may be affected, but the most striking changes seen grossly were in the mesentery where thickened, twisting arteries, beaded by small, aneurysmal dilatations, traversed the tissue. The disease was confined to large and medium-sized arteries. The aorta was never involved and smaller arteries and arterioles rarely.

On microscopic examination, the early phases of periarteritis consisted in the development, within the adventitia of the vessel wall, of eccentric or surrounding foci of inflammation. Lymphocytes, plasma cells, mononuclear and polymorphonuclear leukocytes and sometimes eosinophiles infiltrated the tissue. As the disease advanced fibrous tissue increased, leukocytes accumulated in the media, and the intima was elevated by deposits of fibrin. Later the muscular tissue of the media was destroyed, the elastic tissue broken, the wall irregularly thickened and the lumen narrowed. Thrombi formed to block the lumen or aneurysmal dilatation occurred. In healing, the infiltrating cells disappeared, connective tissue became condensed, thrombi were organized and occasionally canalized, and calcium deposited in the hyalinized wall. All stages of the process sometimes were found in a single artery.

This disease, by interfering with the blood supply, sometimes led to scar formation in the heart, kidneys and other organs, and to hemorrhages into the gut wall, but infarction was not encountered.

Although of itself an inflammatory process, periarteritis of rats was not definitely correlated with other forms of inflammation in this series. Its occurrence was most frequent in a group of the series which had been deprived of either meat or vegetables. Possibly the condition is related to diet, for, in the course of examination of over 400 rats from the colonies of the Wistar Institute, a large proportion of which were over 700 days of age, no evidence of it was seen.[20]

Lesions of the veins were limited to the occasional occurrence of thrombi.

Diseases of the Kidneys. In 9 animals the periarterial inflammation led to considerable scar formation within the renal cortex. Ten other animals developed similar widespread disease of the kidneys, but without arteries being changed. The capsular surfaces of the kidneys in these cases were marked by fine granulations. In sections there was diffuse obliteration of the glomeruli and tubules. Occasionally glomerular capillaries were adherent to the capsule but proliferative changes of the type seen in glomerulonephritis were not frequent.

More frequently, limited areas of fibrosis were formed in the kidneys, about 25% of the series being involved. In these cases rather limited groups of glomeruli became hyalinized, tubules underwent atrophy and fibrous tissue increased. These lesions were of the type which, in other animals, result from gradual stricture of arteries here and there through the renal cortex, usually increasing in severity as age advances and sclerosis of the blood vessels becomes more pronounced. In these rats such an etiology was not established, but it was suggested that renal calculi, of which there was a high incidence, may have pressed upon arteries to produce the change.

Renal calculi also seemed to be the cause of many cases of hydronephrosis which were encountered.

The frequency of renal calculi in this series of rats is to be contrasted with their complete absence in over 400 animals from the colonies of the Wistar Institute.

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